NATURAL ENEMIES AND MORTALITY FACTORS OF THE COFFEE LEAFMINER *Leucoptera coffeella* (GUÉRIN-MÉNÈVILLE) (LEPIDOPTERA: LYONETIIDAE) IN CHIAPAS, MEXICO

A Dissertation

by

J. REFUGIO LOMELI FLORES

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2007

Major Subject: Entomology

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ABSTRACT

Natural Enemies and Mortality Factors of the Coffee Leafminer *Leucoptera coffeella* (Guérin-Ménèville) (Lepidoptera: Lyonetiidae) in Chiapas, Mexico. (December 2007)

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Through field surveys and laboratory experiments, this study assessed in part the impacts of host plant, natural enemies, and weather variables on coffee leafminer *Leucoptera coffeella* distribution and abundance at two elevations and two rainfall levels in coffee farms in Chiapas, Mexico. In addition, a checklist of Neotropical coffee leafminer parasitoids was assembled from field collections and literature review. Coffee leafminer field incidence was positively correlated with leaf nitrogen content and age, but in laboratory experiments coffee leafminers grew larger, developed faster, and had higher survivorship on leaves with moderate (2.9±0.01%) versus low (2.5±0.04%) or high (3.4±0.01%) nitrogen level, and on tough versus soft leaves. Ovipositional preference was not generally for leaves that maximized offspring performance. Coffee leafminer incidence was higher during the rainy versus dry season, and at low versus high elevation. Shade cover reduced ambient temperatures within coffee farms, but did not significantly affect coffee leafminer incidence.

The coffee leafminer predator complex included 16 morphospecies, ~88% of them ants (Formicidae), and contributed >58% of real mortality. Predation rates were higher at high versus low elevation, and under high versus low rainfall. Predation was the main source of coffee leafminer mortality throughout the year, and was highest during the rainy season, when coffee leafminer incidence was highest.

Neotropical coffee leafminer parasitoids included 23 species of Eulophidae and seven of Braconidae. In Chiapas, 22 larval parasitoid morphospecies were collected. Egg and pupal parasitoids were not recovered. Parasitism accounted for <10% of real mortality, and rates were 8-10-fold higher at low versus high elevation. Parasitism rates were not significantly influenced by temperature or rainfall.

Coffee leafminer oviposited mostly during the night, and less under low versus high temperatures. Average monthly temperature minima, which occur during the night, were generally lower at high (~18 ^{o}C) versus low (~20 ^{o}C) elevation farms. The incidence and abundance of coffee leafminer may differ between elevations due to differences in temperature, because at high elevation lower temperatures likely reduced coffee leafminer oviposition, and may have increased its mortality rate as a consequence of longer development time and exposure to natural enemies.

ACKNOWLEDGMENTS

I thank Dr. Julio S. Bernal for his permanent support, patience, and friendship. These years in his lab were full of new knowledge and ways to improve not only my academic performance but also the way to do the things right.

I also want to thank my committee members: Dr. James Woolley, Dr. Robert Wharton, Dr. Roel Lopez, Dr. Juan F. Barrera, and Dr. Carlos Bográn for their guidance and support throughout my research. I thanks my friends and colleagues at the Bernal Lab: Andrea Joyce, James Butler, Veronica Manriquez, Rodrigo Diaz, Aldo Gardea, Brad Hopkins, Melissa Layton, and especially Pat and Alan Gillogly for their help, support and friendship. I would like to say thank you to all my classmates, colleagues, staff, and faculty in the Department of Entomology for making my time at Texas A&M a great experience.

I thanks my friends and colleagues at El Colegio de la Frontera Sur (Joel Herrera M., Enrique López P., Román Montes, Guillermo López G., and Heber García) for their help during the development of experiments in Chiapas, Mexico. I also thank Federico Ochoa (Alpujarras), Alejandro Alvarez (La Gloria), Humberto Prada (El Encanto), Joaquín Erhlich (Lindavista) for permission granted to work on their farms.

I thanks “Centro de Investigación y Desarrollo de la Caña de Azúcar” (Tuxtla Chico, Chiapas, Mexico), for the climatic data provided. I also thank CONACyT (Grant 91539) and Colegio de Postgraduados for funding my research. Thanks to Dr. Eduardo Aranda Delgado (Terranova Lombricultores, Xalapa, Veracruz) for lending me his

coffee leafminer parasitoid collection. I thanks Dr. James Woolley and Dr. Robert Wharton (TAMU) for their help in the identification of coffee leafminer parasitoids, and to Dr. Jorge Valenzuela (Instituto de Ecologia, Xalapa, Veracruz, Mexico) for the identification of ants.

I thanks the editors of Bulletin of Entomological Research, The Canadian Entomologist, Proceedings of the Entomological Society of Washington, Memoirs of the American Entomological Institute, CAB International, Florida Entomologist, NRC Research Press (Canada), International Society of Hymenopterists, and to Mr. Lonny D. Coote (Wildlife Enforcement Division, CWS Environment Canada), and Drs. Chao-Dong Zhu (Institute of Zoology, Chinese Academy of Sciences), Gordon Gordh, (Center for Plant Health Science & Technology, USDA, APHIS, USA), Michael E. Schauff (Plant Sciences Institute Beltsville, MD, USA), Christer Hansson (Lund University, Sweden), John La Salle (Australian National Insect Collection), Mike Gates (Natural Museum of Natural History, Smithsonian Institution, USA), Eduardo Mitio Shimbori (Universidade Federal de Säo Carlos, Brazil), and Alejandro Gonzalez (Universidad Autónoma de Nuevo León) for the permission granted to use the figures in chapter IV.

I also wish to express my gratitude to my parents, Rigoberto and Josefina, my brothers, sisters and their families, whose support was unconditional throughout all my life. Finally, but not least, I would like to say thank you hundreds of times to my wife (Elena Vera-Villagran) and my little daughter (Elenita Lomeli-Vera) for their patience, encouragement, and support during these years living together in the United States, and for making my life full of love.

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CHAPTER I

INTRODUCTION

Integrated Pest Management (IPM) programs are used worldwide to minimize losses in annual and perennial crops. IPM programs rely on combinations of pest control tactics, including host plant resistance, cultural practices, physical control, mechanical control, biological control, and, only when absolutely necessary, chemical control (Odour & Simons, 2003). IPM program design is based upon the biology and ecology of the crop plant, and its herbivore pests and their natural enemies. Thus, one important step in IPM program design is to seek generalizations concerning the effects of different environmental factors on pest and natural enemy population changes in order to explain spatial and temporal patterns of pest abundance. In addition, studies focusing on the effects of environmental and host plant factors on pest mortality and reproduction provide information useful for improving our understanding of pest abundance patterns. In the case of leafmining insects, Auerbach *et al.* (1995) cited three major sources of mortality: vertical sources, including natural enemies and host-plant attributes; horizontal sources, such as intra- and inter-specific competition, and; abiotic sources, which include meteorological factors, such as wind, storms, frosts, strong rain, moisture, and extreme temperatures. In this context, the main objective of the present study was to assess the impact of coffee plant characteristics, natural enemies, and weather variables

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This thesis follows the style and format of the *Bulletin of Entomological Research*.

on coffee leafminer *Leucoptera coffeella* (Guérin-Ménèville) (Lepidoptera: Lyonetiidae) mortality, population dynamics, and spatial distribution in coffee farms in the Soconusco region of Chiapas, Mexico. Coffee leafminer is an important pest of coffee in the Americas, particularly in areas where coffee is grown under full-sun conditions (see below).

Nearly 100 species of coffee (*Coffea* L.) are known (Coffee and Conservation, 2006), but only three are used in commercial coffee plantations, *Coffea arabica* L., *C. canaphora* Pierre ex-Froehner, and *C. liberica* Bull ex–Hiern. *Coffea arabica* (arabica coffee) is the most important species, accounting for >70% of world production (International Coffee Organization, 2005). Coffee is the main agricultural export crop in many developing countries, and is an important component of the Mexican agricultural economy, particularly in the southern states of Chiapas, Oaxaca, and Veracruz (Consejo Mexicano del Café, 2002). Coffee yields and quality are directly and indirectly influenced by both abiotic and biotic factors. Enriquez (1993) reported that optimal weather conditions for growing arabica coffee included temperatures between 18 and 21 ºC, annual rainfall between 1200 and 1800 mm with a dry season not exceeding 3 months, and relative humidity between 70 and 95%, though high humidity increases disease and pest risks. Also, Haggar *et al*. (2001) suggested that shade cover between 40 and 60% maximizes production, and reduces disease and pest risks. Such conditions, however, are strongly affected in turn by geography, particularly elevation and topography, total and daily luminosity, and cultural practices (Enriquez, 1993).

Kimani *et al.* (2002) cited >900 insect species feeding on coffee worldwide, and Odour and Simons (2003) cited >1420 arthropod species inhabiting traditionally managed coffee agroecosystems, though very few were important coffee pests, and >750 were entomophagous species. Worldwide, the coffee berry borer *Hypothenemus hampei* (Ferrari) is considered the main coffee pest (Bustillo *et al.,* 2002; Barrera *et al.,* 2000), though changes in crop management, particularly reductions of shade cover and increases of pesticide use in coffee farms, have led to major outbreaks of secondary pests, including coffee leafminer (Guharay *et al.,* 2001; Monterrey *et al.,* 2001; Fragoso *et al*., 2002; Carvalho *et al*., 2005). In particular, shade cover reduction and increased pesticide use were believed to lead to reductions in coffee leafminer natural enemy populations, and consequently to increases in coffee leafminer populations (Fragoso *et al.*, 2002), though this was not closely examined.

Following the emergence of coffee leafminer as a major pest in some New World coffee producing countries, such as Brazil, Colombia, Cuba, Guatemala, Peru, and Puerto Rico, researchers focused their attention on understanding its biology (Enriquez *et al.,* 1975; Parra, 1985; Fernándes *et al.,* 2004) and effects of host plant resistance (Guerrero-Filho & Mazzafera, 2000; Guerreiro-Filho, 2006), its natural enemies (Parra *et al.,* 1977; Flórez & Hernández, 1982; Aranda-Delgado, 1986; Gallardo, 1988; Pereira *et al.,* 2003), and on developing environmental management tactics (Fernández, 1978; Gravena, 1983; Bustillo & Villacorta, 1994, Fragoso *et al.,* 2001, 2002). The sections that follow summarize the most relevant research findings concerning coffee leafminer,

and identify IPM-related research needs specific to the Soconusco area of Chiapas, Mexico.

Coffee leafminer distribution and biology

Coffee leafminer is cited in most literature as *Leucoptera coffeella*, while in the Brazilian literature it is referred to as *Perileucoptera coffeella* (Guérin-Ménèville). *Perileucoptera* is a genus proposed by F. Silvestri in 1943, which has not been accepted as the valid name (Green, 1984). Coffee leafminer was described from specimens collected in the Antilles, but some workers suspected a foreign origin because native host species were absent in the New World. Green (1984), based on a series of elements, proposed that the Reunion Island in Africa is the native home of *L. coffeella*. Coffee leafminer is a species with tropicopolitan distribution, but is an important pest only in the Neotropics (CAB International, 2000). Although important outbreaks of coffee leafminer were recorded from East Africa in earlier literature, Bradley (1958) showed that those outbreaks were in fact due to *L. meyricki* Ghesquiere, and it appears that *L. coffeella* is uncommon in the Old World (CAB International, 2000). The first report of *L. coffeella* in the Americas was in 1842 from the Antillean region, followed by Brazil in 1850, after which it was found in most American coffee producing countries (Green, 1984).

Coffee leafminer is a nocturnally active species (Mendoza, 1995), whose larvae are known only from *Coffea* spp. leaves (Ramiro *et al*., 2004). Adults are small, silver moths (~3 mm long) (Mendoza, 1995), have a 2-3 week life span, and, depending on the

temperature, 4-12 generations per year (Parra, 1985). Females lay 10-60 eggs, typically 3 to 7 per leaf, on the upper surface of coffee leaves (Bustillo & Villacorta, 1994). Parra (1985) found that 27 ^{o}C was the ideal temperature for coffee leafminer development and oviposition, and that at this temperature eggs hatched in ~5 days. First-instar larvae initiate mines by penetrating the leaf epidermis through the lower part of the egg, which is in contact with the leaf epidermis, and feed on palisade parenchyma cells (Ramiro *et al*., 2004). Thus, larvae are minimally exposed to the leaf surface and environment outside the leaf (Bustillo & Villacorta, 1994). Coffee leafminer larvae remain in the mine for ~10 days (Parra, 1985). Several mines may coalesce, so several larvae may be found in a single mine. The last instar larva (~5 mm in length) leaves the mine to spin a cocoon (~6 h) on the underside of a coffee leaf of the lower part of the coffee plant; this instar is the only one which is exposed to the external environment (Enriquez *et al*., 1975). Coffee leafminers spend ~6 days in the pupal stage, after which they emerge as adults, mate, and lay eggs (Parra, 1985; Rosales-Mondragón *et al*., 2003). In severe infestations, coffee plants are defoliated and suffer yield loss, which may exceed 50%, such as occurred in Brazil and Guatemala (Fernández, 1978; Paliz & Mendoza, 1993).

Host plant effects on coffee leafminer

Host plant characteristics, such as leaf penetrability, leaf age, leaf surface area, availability of young leaves, leaf abscission, chemical composition, nutritional quality, moisture content, and leaf water potential, have been suggested as important factors influencing herbivore host preference, oviposition success, and egg distribution patterns

(Hespenheide, 1991; Leather, 1994; Wheeler & Center, 1996; Scheirs, 2002; De Bruyn *et al*., 2002; Scheirs *et al*., 2004; Forister, 2005; Heisswolf *et al.,* 2005; Guerreiro-Filho, 2006), as well as survival and eventual fecundity of a herbivores' offspring (Leather, 1995; Klingenberg & Spence, 1997; Awmack & Leather, 2002; Moreau & Benrey *et al.*, 2006). Some studies suggested that leafminer females, including coffee leafminer, select host plants for oviposition on the basis of plant defensive attributes, vegetational background, microclimate, and presence of other organisms, such as competitors and natural enemies, among other factors (Hespenheide, 1991; Valladares & Lawton, 1991; Leather, 1994; Wheeler & Center, 1996; Scheirs, 2002; De Bruyn *et al*., 2002; Scheirs *et al*., 2004; Forister, 2005; Heisswolf *et al.,* 2005; Guerreiro-Filho, 2006). Guerreiro-Filho (2006) reviewed the information available concerning coffee host plant resistance to coffee leafminer, and suggested that leaf age, size, toughness, and nitrogen content contributed to coffee leafminer mortality and fecundity. Preliminary surveys conducted in coffee farms in Chiapas, Mexico, showed a high variability in coffee leafminer incidence among plants within individual coffee farms (JRLF, unpubl. data). Such variability may be due to genetic variation in host plant characteristics, micro-environmental conditions, and/or pressure from natural enemies. The second chapter of this dissertation describes a study conducted in Chiapas, Mexico, consisting of a series of field surveys and laboratory experiments designed to assess whether coffee leaf quality parameters influence host plant selection and fitness components in coffee leafminer.

Coffee leafminer natural enemies

Coffee leafminer natural enemies have been studied in Brazil (Parra *et al.,* 1977; Souza, 1979; Mendoza, 1995; Fragoso *et al.,* 2001; Fernández & Bueno, 2002), Colombia (Flores & Hernández, 1982; Mendoza, 1995), Peru (Enriquez *et al.,* 1975), Ecuador (Mendoza, 1995), Cuba (Konnorova, 1987), Puerto Rico (Gallardo, 1988; Mendoza, 1995), and Mexico (Aranda-Delgado, 1986). Overall, the list includes at least 10 predatory wasps (Vespidae), 21 Eulophidae and seven Braconidae primary larval parasitoids. In Mexico, only Aranda-Delgado (1986) studied the natural enemy complex of coffee leafminer. He reported 14 genera and four species from Veracruz, and nine genera and two species from Chiapas. However, Aranda-Delgado's collections from Chiapas were sporadic, and focused exclusively on parasitoids. Thus, the coffee leafminer parasitoid complex for Chiapas as reported by Aranda-Delgado (1986) is likely incomplete, and the predator complex has thus far not been studied. Currently, catalogs and/or taxonomic identification keys for coffee leafminer parasitoids are not available, and the lack of such documents slows down studies on coffee leafminer parasitoids, and biological control and management of this pest. In addition, substantial changes in the nomenclature of various parasitoid taxa associated with coffee leafminer have been made in recent years, thus some records need to be updated. Therefore, in Chapter IV of this dissertation an annotated checklist of coffee leafminer parasitoids was compiled, and a key for identifying the genera of parasitic Hymenoptera associated with coffee leafminer in the Neotropical region was constructed. The goal of Chapter IV was to summarize the information available in the literature on coffee leafminer parasitoids,

including recent nomenclatural changes, and to update the known coffee leafminer parasitoid complex for the Neotropics through collections in Chiapas, Mexico.

Previous studies on coffee leafminer population dynamics by natural enemies suggested an important influence of predatory wasps, and showed a reduction of 69% of coffee leafminer populations due to predatory Vespidae (Reis *et al*., 2000). On the other hand, many of the studies published in recent decades suggested an important impact of parasitoids on coffee leafminer populations (Villacorta, 1980; Flórez & Hernández, 1982; Aranda-Delgado, 1986; Gallardo, 1988), though few detailed studies are available. For example, the larval parasitoid *Mirax insularis* Muesebeck (Braconidae) was introduced to Puerto Rico where it provided partial biological control of coffee leafminer (Gallardo, 1988). Also, Mendoza (1995) reported 80% coffee leafminer parasitism by *M. insularis* in the islands of Guadalupe and Dominica. Finally, some authors noted strong negative impacts of shade cover reduction and extensive pesticide use on coffee leafminer natural enemy populations, though most studies did not quantify such impacts (Guharay *et al.,* 2001).

Many studies quantifying coffee leafminer mortality due to natural enemies focused exclusively on larval mortality (e.g., Parra *et al*., 1977; Villacorta, 1980; Aranda-Delgado, 1986; Reis *et al*., 2000), though preliminary studies conducted in Chiapas, Mexico, suggested that coffee leafminer eggs and pupae suffered higher mortality rates due to predation than coffee leafminer larvae, and that mortality rates at all developmental stages were affected by the environmental conditions prevalent at different elevations (JRLF, unpubl. data).

Investigating in detail the natural enemy complex associated with coffee leafminer of all developmental stages, and its impacts on coffee leafminer population dynamics should provide important information, such as the identities of key mortality factors and natural enemies important for managing this pest. Studies described in chapters III and IV of this study sought to identify the natural enemy complex associated with coffee leafminer, and to contrast the main mortality factors affecting the population dynamics of coffee leafminer at two elevations in Chiapas, Mexico.

Effects of weather on coffee leafminer

Coffee leafminer outbreaks in Brazil were sporadic through 1970 when most coffee was grown under shade conditions, but their intensity and frequency increased dramatically with the introduction of more productive coffee varieties that required greater use of agrochemicals and full sun conditions (Fragoso *et al*., 2002). Moguel and Toledo (1999) noted that elimination of shade cover in coffee farms led to a less stable physical environment, and lower microorganism abundance and diversity. As a general rule, the incidence of coffee leafminer in the Neotropics was associated with low rainfall and high temperature (Reis et al., 1976; Villacorta, 1980; Gravena, 1983; Nestel, *et al*., 1994; Costa-Conceiçao, 2005). In tropical environments, monthly average temperatures typically vary minimally during the year, thus, some authors suggested that rainfall, which is more variable, was the main source of coffee leafminer adult and larval mortality, particularly during the rainy season (Villacorta, 1980; Campos *et al*., 1989). However, such suggestions were based on the dynamics of mined coffee leaves in the

field, rather than direct evaluation of coffee leafminer densities and mortality sources. In addition, Guharay *et al*. (2001) suggested that increases in coffee leafminer populations in farms where shade was reduced may be related to changes in micro-environmental conditions that directly or indirectly affect coffee leafminer developmental time or mortality due to natural enemies. However, the effects of abiotic factors on both coffee leafminer and its natural enemies were not evaluated in detail. The study described in chapter V of this dissertation assessed through field surveys and laboratory experiments the effects of temperature, rainfall, and shade cover on seasonal survival rate, distribution, and abundance of coffee leafminer and its natural enemies at two elevations in Chiapas, Mexico.

Dissertation objectives and goals

To date, most available studies addressing the impacts of host plant attributes, natural enemies, or weather conditions on coffee leafminer population dynamics (see above for references) focused on the larval stage, few of them explored more than one source of mortality at once, and none explored mortality sources affecting the egg and pupa stages. Consequently, few data are available concerning the impacts of mortality sources on all coffee leafminer developmental stages, and the impacts of those mortality sources on coffee leafminer population dynamics under variable weather conditions and elevations.

The overall goal of the research described in this dissertation was to contribute to the development of IPM strategies for coffee leafminer in the Soconusco region of Chiapas, Mexico. Specifically, the objectives of the research were to:

1) Assess whether selected coffee leaf quality parameters (age, size, nitrogen content, penetrability index, specific weight, and presence of previous damage) affect coffee leafminer host plant selection and fitness components;
2) Identify and catalogue the natural enemies associated with coffee leafminer in the study area, and;
3) Quantify and compare the main mortality factors affecting the population dynamics of coffee leafminer at two elevations and two rainfall levels in the study area.

CHAPTER II

HOST PLANT EFFECTS AND SELECTION IN A MONOPHAGOUS INSECT: COFFEE LEAFMINER *Leucoptera coffeella* (GUÉRIN-MÉNÈVILLE) (LEPIDOPTERA: LYONETIIDAE)

Introduction

Plants defend themselves against herbivores through chemical and physical defenses, and herbivores have evolved strategies to counter or avoid the negative impacts of such defenses. For example, herbivore strategies include avoiding host plants with low nutritional value or that have negative physiological effects on them or their offspring. Thus, host plant selection is a critical process for herbivorous insects, especially in those where the larval stage has low mobility, and is affected by many factors, including plant defensive attributes, vegetational background, microclimate, and presence of other organisms, such as competitors and natural enemies, among other factors (Valladares & Lawton, 1991). A number of studies showed that host plant characteristics, such as availability of young leaves, and leaf penetrability, age, area, shape, surface structures, abscission, chemical composition, nutritional quality, and water potential influence herbivore host preferences, oviposition success, and egg dispersion patterns (Hespenheide, 1991; Leather, 1994; Wheeler & Center, 1996; De Bruyn *et al*., 2002; Scheirs *et al*., 2004; Forister, 2005; Heisswolf *et al.,* 2005; Guerreiro-Filho, 2006), as well as survival, adult and larva size, and eventual fecundity of offspring (Leather, 1994; Klingenberg & Spence, 1997; Awmack & Leather, 2002; Moreau &

Benrey, 2006). Host plant selection by insects thus likely influences the distribution and dynamics of insect populations (Auerbach *et al.,* 1995; Hopkins & Memmott 2003; Karimzadeh *et al.,* 2004), including populations of species that are agricultural pests.

At least three hypotheses have been proposed to explain why herbivore insects choose certain plants over others to lay eggs. The *optimal oviposition behavior hypothesis* predicts that herbivore insects preferentially oviposit on plants where their offspring have access to an optimal supply of food (Jaenike, 1978); this hypothesis is particularly relevant to sessile insects, such as leafminers, whose larvae are largely incapable of searching for hosts. *The optimal foraging hypothesis* predicts that females of species whose adults and larvae feed from the same host plant select high quality hosts for adult feeding in order to maximize the number of eggs laid; therefore, eggs frequently are laid on plants that are suboptimal for offspring development (Jaenike, 1986; Stephens & Krebs, 1986; Scheirs *et al*., 2004). Predictions of the optimal foraging hypothesis are particularly likely in insects with wide host ranges, highly mobile larvae, and adults that feed on the host plant, such as some Agromyzidae leafminers (Scheirs, 2002; Lamb *et al*., 2003; Forister, 2005). The *enemy-free space hypothesis* predicts that herbivores will oviposit on low quality host plants when these provide natural enemy-free space for their offspring (Jefferies & Lawton, 1984; Hawkins *et al*., 1997; Moon & Stiling, 2006). Leafminers commonly support a highly diverse complex of natural enemies (Hespenheide, 1991; Whitfield & Wagner, 1991; Schauff *et al*., 1998), so the enemy-free space hypothesis may explain in part how leafminer adults select their host plants.

A number of studies on lepidopteran insects support the optimal oviposition behavior hypothesis on the basis of significant correlations between host plant quality and herbivore performance (Rausher, 1979; Stamp & Bowers, 1990; Berdegué *et al*., 1998, and references therein). For instance, the optimal oviposition behavior hypothesis may generally explain the abundance and distribution of monophagous species with sessile larvae, such as coffee leafminer *Leucoptera coffeella* (Guérin-Ménèville) (Lepidoptera: Lyonetiidae). In contrast, other studies showed that lepidopteran insects did not always oviposit on plants where offspring performance was optimized (Mayhew, 1997; Berdegué *et al*., 1998; Forister, 2005).

Coffee leafminer is an important pest of coffee (*Coffea arabica* L.) in the New World, including Mexico (CAB international, 2000; Ruíz *et al*., 2004). Coffee leafminer life history, feeding habits, and local distribution patterns are in line with assumptions of the optimal oviposition behavior hypothesis, as suggested for leafminers (Scheirs, 2002). First, coffee leafminer incidence is highly variable among and within coffee farms in Chiapas (see chapters III and V), suggesting that the suitability of coffee plants for coffee leafminer development is variable. Second, coffee leafminer larvae are known only from *Coffea* species, obligatorily develop within the leaf selected for oviposition by the parental female, and leave that leaf only to pupate (Parra, 1985; Aranda-Delgado, 1986; Ramiro *et al*., 2004). Finally, adult coffee leafminers are nectar feeders, and are not known to obtain protein from host plants (Walker & Quintana, 1969; Guerreiro-Filho, 2006). These characteristics suggest that relevant aspects of coffee leafminer's host selection process may be predicted by the optimal oviposition behavior hypothesis.

Testing whether optimal oviposition behavior predictions are borne in coffee leafminer not only helps to understand its host-plant selection process, but also may yield information useful for its management.

Studies addressing host plant selection by coffee leafminer are not available, despite their potential importance for developing management tactics against this pest. Previous studies showed variation in some coffee plant characteristics, including leaf size, penetrability, and nitrogen content, associated with fertilization regimes and shade levels (Stamp & Bowers, 1990; Castillo *et al*., 1997; Blanco *et al*., 2003; Forister, 2005; Baumann, 2006; Guerreiro-Filho, 2006). Such variation may partially explain evident variation in coffee leafminer incidence within and among coffee farms.

The present study evaluated likely factors influencing the distribution and abundance of coffee leafminer within and among coffee farms, according to predictions of the optimal oviposition behavior hypothesis. Specifically, the study addressed whether (i) variation in specific coffee leaf characteristics (nitrogen content, size, and penetrability indices) was related to the incidence of coffee leafminer in coffee farms, and (ii) coffee leafminer oviposition preference and fitness components (adult and larva size, egg and larval survival, and development time from egg to pupa) were influenced by coffee leaf characteristics (size, age, nitrogen content, and presence of coffee leafminer larvae) in the laboratory. These coffee leaf characteristics were selected on the basis of prior studies on coffee leafminer and other lepidopteran species (Castillo *et al*., 1997; Forister, 2005; Guerreiro-Filho, 2006).

Materials and methods

Field surveys and laboratory experiments were conducted to assess variability in coffee plant characteristics and coffee leafminer incidence in a coffee farm (*La Gloria*, 15^{o} 00' 07'' N; 92^{o} 09' 24'' W; 550 m, ~70 ha) in Cacahoatán, within the Soconusco region of Chiapas, Mexico, and whether various coffee plant quality attributes affected host plant selection and fitness components in coffee leafminer. The main coffee varieties in the coffee farm were *Borbon*, *Typica*, and *Catuai*, but all studies involved *Catuai* plants. Insecticides were not applied to plants in the farm during the study period. All laboratory experiments were conducted at El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico.

Coffee leafminers used in experiments were reared on detached coffee leaves as described in Reis *et al*. (2000), in a rearing room at 26±1 ^{o}C, humidity close to saturation, and a natural photoperiod (~13L: 11D). A coffee leafminer colony was started with field-collected larvae one month before the experiments were initiated. Coffee leafminer pupae from the lab colony were held individually in 5 ml glass vials to obtain virgin adults of known age for experiments. Upon emergence, coffee leafminer adults were sexed and provided with two small droplets of pure honey inside the glass vial, and then used in experiments. Coffee leafminer females and males were held together in a glass vial for 24 h to facilitate mating before using them in experiments.

Throughout, data recorded as proportions (mined leaves per plant, survivorship) were arcsine $\sqrt{x}$-transformed for analyses. Before analyses, all data were tested for conformity to requirements for parametric analysis. Means (± standard errors) are

presented in figures, unless indicated otherwise. All statistical analyses (see below) are described in Sokal and Rohlf (1995) and/or Zar (1996), and were conducted using SPSS 12.0 (SPSS, 2005, Chicago, Illinois, USA.), with α set at 0.05.

Variation in coffee leafminer incidence among and within coffee plants. Variation in coffee leafminer incidence, scored as proportion mined leaves per plant, was assessed via a survey in late spring through summer of 2005, when coffee leafminer incidence typically is greatest in this area (see chapter V). In April, 30 plots consisting of four adjacent plants were selected at random, though avoiding 10 rows or coffee plants from the edges. Eight branches, each with eight leaves free of mines were marked per each plant (= 960 branches, 7680 leaves) at the beginning of the survey (April) to quantify between-plant variation in leafminer density. Proportion mined leaves per plant was estimated one (May) and four months (August) later. Mined leaves included only leaves with fresh mines, i.e. those containing either a living or newly dead coffee leafminer larva. Kurtosis indices were estimated to characterize variability in coffee leafminer incidence among coffee plants within sampling dates. Relationships were sought between proportion mined leaves per plant, a proxy for coffee leafminer incidence, and coffee leaf nitrogen content or leaf size (see below).

Coffee leafminer incidence on leaves of different ages within coffee plants was assessed using leaf position on a branch as an index of leaf age, since older leaves are positioned at the base of the branch while younger leaves are at the branch tip (Guerreiro-Filho, 2006). One branch with at least one leaf with a fresh mine was sampled from 150 randomly selected plants in August 2005. Ten pairs of leaves per

branch, beginning at the apical pair, were examined for mines, and presence/absence of mines was recorded for each leaf pair. The frequencies of mined leaves among the 10 pairs were subjected to a Chi-square goodness of fit test, followed by a Tukey-type multiple comparisons test for proportions (Zar, 1996). The null hypothesis was that mined leaves were distributed independently of position (1-10) on a branch.

Coffee leafminer incidence, and larva and adult size in relation to leaf quality. Leaf nitrogen content was evaluated for one plant per plot at each of the 30 plots (see above, *Variation in… within coffee plants*) at the beginning of the study (April, 2005). Thirty undamaged leaves from the second apical pair on a branch were collected from each plant and immediately transported in a cooler to a local commercial laboratory (Laboratorio Agroecológico Terra, Tapachula, Chiapas) for leaf nitrogen analysis. In addition, variability in nitrogen content among leaf positions (≈ age) within branches was assessed from 12 randomly selected plants. Thirty undamaged leaves per plant from each of the first, third, and fifth pairs, representing three different ages within a season (Blanco *et al*., 2003), were sampled from each of the 12 plants. Leaf nitrogen content was determined in the local commercial laboratory using the Kjeldahl method for organic nitrogen (Broadstreet, 1965). Monge (1999) indicated that 2.5 to 3.5% was the optimal leaf nitrogen content for coffee, and suggested that percentage leaf nitrogen was a good indicator of the nutritional status of coffee plants. The proportion of mined leaves per plant was estimated for each of the 30 plants by dividing the total number of mined leaves by the total number of leaves on the eight labeled branches. Regression analysis was used to uncover any relationship between proportion of mined leaves and

leaf nitrogen content per plant. ANOVA and Tukey's post-hoc tests were used to compare leaf nitrogen content among leaf ages within branches.

Leaf size effects on coffee leafminer incidence observed in May and August 2005 were assessed using 10 undamaged leaves from second apical pairs from each of the 960 branches labeled in April (see above, *Variation in… within coffee plants*). Leaf size was determined using leaf length as a proxy for leaf surface area. A preliminary sample from the same locality, consisting of 100 leaves collected in April 2005, showed a strong correlation between leaf length and leaf surface area ($P < 0.001$, $r^2 = 0.954$), so the corresponding relationship, $surface\ area = 1.57 - 0.53length + 0.34length^2$, was used to estimate leaf surface area from length measurements. The relationship was validated using a sample of 100 coffee leaves collected in the same coffee farm ($r^2 = 0.96$, $P = 0.001$). Regression analysis was used to uncover any relationship between proportion of mined leaves and leaf size per plant.

Leaf penetrability and specific weight effects on coffee leafminer larva size were assessed using 300 field collected leaves with active mines. Mined leaves were individually labeled, taken to the laboratory, and held in 0.5 l plastic storage bags until coffee leafminer larvae emerged to pupate. Larvae were collected when they emerged from mines to pupate and were stored in 5 mm vials with 80% alcohol. Coffee leafminer larva head capsule width was measured under a dissecting microscope at 25× magnification using an eyepiece micrometer with 0.01 mm resolution. Leaf penetrability index, a measure of leaf hardness (Moran & Buchan, 1975), and leaf specific weight, a measure of leaf toughness (Foss & Rieske, 2003), were assessed in leaves from which

coffee leafminer emerged to pupate. A leaf penetrability index was assessed using a mechanical fruit firmness tester (portable penetrometer model FT-01, QA Supplies LLC, Norfolk, Virginia, USA) with a 2.0 mm tip. Detached leaves were held firmly between two acrylic glass plates with 4 mm holes as guides for the insertion of the penetrometer tip. Five measurements of leaf penetrability were made per leaf, avoiding leaf veins and the median measurement was used for analyses. Leaf specific weight (mg/cm^2) was determined from the apical portion (~3 cm) of leaves (without mined tissue), which was scanned and dried to constant weight at 60 ^oC for 48 h. The apical portion surface area of each leaf was measured using Sigma Scan Image Analysis 2.0 software (Jandel Scientific, Corte Madera, California, USA), and dry weight using a microbalance. The potential relationships between coffee leafminer larva head capsule width versus leaf penetrability index (n = 192 leaves) or leaf specific weight (n= 172 leaves) were examined via lineal regression analyses.

Coffee leafminer oviposition preference in relation to leaf quality.

Oviposition preferences in relation to leaf size (surface area), age (position on branch), and nitrogen content, and presence of coffee leafminer larvae were evaluated in the laboratory using field collected leaves and laboratory reared coffee leafminer adults. Coffee leaves from the second apical pair in a branch, and of similar size were used in all oviposition preference experiments to reduce variability among leaves, except when leaf age and leaf size were the variables under evaluation (see below). Detached leaves were rinsed in tap water prior to trials to remove dust and other contaminants that could interfere with coffee leafminer oviposition. After air-drying, six leaves (one or two per

each variable under evaluation) were randomly arranged in a 10 cm–diam circle on a sponge saturated with water in a 4 l plastic cage. Organdy fabric in the top and bottom of the cage allowed aeration. Leaves were labeled according to the treatment variable, and leaf position in the circle (1, 2, 3, etc.) was recorded. One pair of coffee leafminer adults was released inside each cage, and the females were allowed to freely oviposit during 24 h. The number of eggs per leaf was recorded after this time. New adults were used in each trial, and each coffee leafminer pair was considered a replicate. Twenty replicates were conducted for each leaf quality variable. However, only trials in which eggs were present after 24 h were included in analyses: 13 trials for leaf size, 15 for leaf age, 17 for nitrogen content, and 18 for presence of coffee leafminer larvae.

Oviposition preference in relation to leaf size was assessed using leaves of three size categories, "small," "medium," and "large." Leaf age was controlled by using only leaves from the second pair on a branch. All leaves in each trial came from a single coffee plant, but from different branches. Size categories were defined prior to the trials by collecting 100 second-pair leaves from the field and estimating their surface area (see above *Coffee leafminer incidence...leaf quality*). The 100-leaf sample included leaf sizes between 5.4 and 93.6 cm^2, with a mean of 35.1±3.9 cm^2. Three size categories, each falling within a 33% percentile, were defined as follows: small = 11 to 25 cm^2 (average = 19.0±0.7 cm^2), medium = 27 to 42 cm^2 (34.9±0.9 cm^2), and large = 43 to 68 cm^2 (56.5±1.5 cm^2). Within each trial, there was a difference of at least 5 cm^2 among leaves corresponding to the leaf size categories.

Oviposition preference for leaf age was tested using the six apical pairs of leaves from one branch (one leaf per pair) of one plant. All branches were taken from a single plant. Leaf position on the branch was used as an index of leaf age.

Oviposition preference for leaf nitrogen content was tested using leaves from the 30 plants previously sampled in the field survey (see above, *Coffee leafminer incidence… leaf quality*). The 30 plants included leaf nitrogen contents between 2.4 and 3.6% of dry weight, and had a mean of 2.9±0.1%, and most plants had nitrogen levels within the optimal range suggested by Monge (1999). Three categories of nitrogen content were defined as follows: low, 2.5-2.6% (2.53±0.04%); medium, 2.9-3.0% (2.90±0.01%), and; high, 3.3-3.6% (3.40±0.07%). Four plants per each of the three leaf nitrogen levels were selected for the trials. Two leaves from one plant per each nitrogen level were used in each trial.

The effect of the presence of coffee leafminer larvae on oviposition preference was tested using three pairs of leaves from the same plant. Each pair consisted of one mined and one mine-free leaf from the second pair on the same branch; a different plant was used for each replicate. Leaves of similar size where used in each trial to standardize for leaf size.

Oviposition preference in relation to each leaf quality variable and leaf position in the arena was assessed via the Scheirer-Ray-Hare extension of the Kruskal-Wallis test, using as dependent factor the number of eggs per leaf in all variables, but number of eggs per cm^2 in trials in which leaf size was the variable under study.

In addition to the above laboratory experiments, oviposition preference in relation to leaf age (position on a branch) was assessed on coffee plants in the field. Ten coffee plants (= replicates) were randomly selected in the coffee farm, and two branches with the apical three pairs of leaves free of mines were selected from each plant. Then, each branch was covered with a 30 cm organdy fabric sleeve. Two pairs of coffee leafminer adults (24 h-old) were enclosed in each sleeve for 48 h, and after this time the number of eggs laid per leaf was recorded. The experiment was repeated four times on different dates (June 7, 9, 16, July 23, 2005). The number of eggs per leaf age and date were compared via the Scheirer-Ray-Hare extension of the Kruskal-Wallis test.

Coffee leafminer survival, size, and development time in relation to leaf nitrogen content and penetrability index. Coffee leafminer adult and larva size were used as fitness proxies for the purpose of this study. Previous studies showed a significant correlation ($P<0.001$, $r^2 = 0.595$) between coffee leafminer size and total fecundity (JRLF, unpubl. data), and other studies showed similar relationships in other Lepidoptera species (Miller *et al*., 1982; Tammaru *et al*., 1996; Van Dongen *et al*., 1999; Catta-Preta & Zucoloto, 2003). Specifically, in this study the length of the adult hind tibia (LHT) and width of the last-instar larva head capsule were used as proxies for coffee leafminer adult and larva size, respectively.

The effects of leaf nitrogen content, low, medium, or high, and leaf penetrability index, both as described above, on coffee leafminer survival, development time, and size were evaluated in the laboratory. Laboratory reared coffee leafminer adults were used in all trials. Thirty coffee plants were analyzed for leaf nitrogen level (see above, *Coffee*

leafminer incidence … leaf quality) and 100 plants for leaf penetrability index (see *Coffee leafminer incidence… leaf quality*). A subset of plants with the targeted leaf nitrogen level or penetrability index was selected, and leaves were collected and rinsed in tap water to eliminate contaminants before use in experiments. Individual coffee leaves were placed on a polyurethane sponge saturated with water inside a 0.5 l plastic cage with organdy fabric windows. One female and one male coffee leafminer were placed inside a cage with one coffee leaf and a droplet of pure honey. Females were allowed to lay eggs for 24 h. After this period, coffee leafminers were removed from the cage, and the number of eggs was adjusted to 1-5 per leaf to minimize larval competition.

Leaf nitrogen content. The three levels of leaf nitrogen indicated above were evaluated: low, medium, or high (see above *Coffee leafminer oviposition… leaf quality*). Four plants per nitrogen level, from the 30 plants that were evaluated, were used to compare leafminer survival, development time, and adult size among nitrogen levels; the coffee leafminer eggs on six leaves per plant represented one replicate. Survival from egg to larva, larva to pupa, and pupa to adult, and development time from egg to pupa were recorded for each egg. The LHT of 10 randomly selected adults per replicate was measured to compare adult sizes among nitrogen levels. The effects of leaf nitrogen level on survival, development time, and adult size were assessed via ANOVA. Tukey's HSD procedure was used to compare means among treatments, as appropriate.

Leaf penetrability index. Leaf penetrability index was assessed in the field from one hundred coffee plants randomly selected using a portable penetrometer (see *Coffee*

leafminer incidence… leaf quality). Twenty plants representing different levels of penetrability were selected. Five leaves per each plant were selected to assess the impacts of leaf penetrability on coffee leafminer survival from egg to larva, and from larva to pupa. The penetrability index was assessed for each leaf. Survival from egg to larva and from larva to pupa was assessed using the methodology described above. In addition, the effect of leaf penetrability on coffee leafminer larva size was assessed on 170 coffee leafminer larvae reared on leaves with known penetrability indices. Larvae were collected in 80% alcohol when they left the mine to pupate, and head capsule width was measured under a dissecting microscope at 25× magnification using an eyepiece micrometer with 0.01 mm accuracy. Potential relationships between leaf penetrability and coffee leafminer survival from egg to larva or from larva to pupa, or larval size were examined via linear regression analyses.

Results

Variation in coffee leafminer incidence among and within coffee plants.

Coffee leafminer incidence was highly variable among coffee plants (figs. 2.1a, b). The 120 coffee plants selected in April without coffee leafminer damage, had proportions of mined leaves between 0 to 0.19 at the end of the study, with a mean of 0.04±0.01 in May (fig. 2.1a), and 0.07±0.01 in August (fig. 2.1b). Variability in coffee leafminer incidence across coffee plants was particularly evident in August, when the distribution was near-mesokurtic (Kurtosis = -0.52, standard error = 0.45) versus May, when it was leptokurtic and positively skewed (Kurtosis = 2.68, standard error = 0.45). In August, ~25% of the

plants had a proportion of mined leaves per plant <0.02, ~50% had a proportion of 0.02-0.1, and ~ 25% had a proportion of 0.1-0.19 (fig. 2.1b). Coffee leafminer incidence within coffee plants was also variable. Mines were not uniformly distributed among the first 10 leaf pairs on branches ($P < 0.001$), occurring most frequently on leaves at positions 5 through 10 (oldest leaves), followed by positions 3 and 4, and least frequently at positions 1 and 2 (youngest leaves) (fig. 2.2).

Coffee leafminer incidence, and larva and adult size in relation to leaf quality. Leaf nitrogen content varied among plants (mean = 2.9±0.03%, range 2.4 to 3.6%). Also, significant variation in leaf nitrogen was evident within plants among leaves from the first, third, and fifth pairs on branches ($F_{2,33} = 39.94$; $P <0.001$). Leaves from the first pair had significantly higher nitrogen content (2.52±0.20%) than leaves from the third (2.15±0.15%) and fifth (1.98±0.09%) pairs, and leaves from the third pair had significantly higher nitrogen content than leaves from the fifth pair. The proportion mined leaves per plant was not correlated with leaf nitrogen content at one month ($r^2 = 0.01$; $P = 0.54$) (fig. 2.3a), though it was positively correlated at four months ($r^2 = 0.25$, $P = 0.005$) (fig. 2.3b).

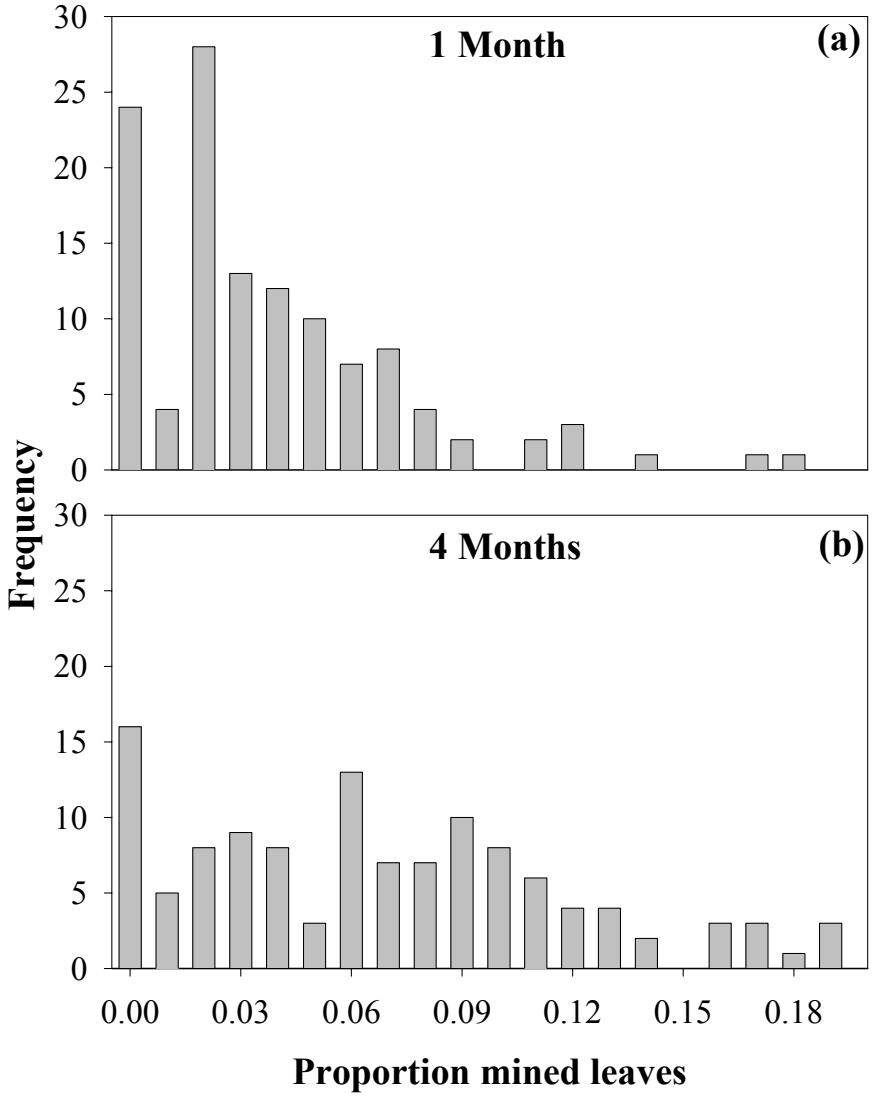

Fig. 2.1. Frequency distribution of proportion of leaves with coffee leafminer mines per eight branches/plant at "*La Gloria*" coffee farm, Cacahoatán, Chiapas, Mexico. Unmined leaves (branches with 64 leaves/plant, 120 plants) were labeled in April, 2005, and examined for mines after (a) 1 month (May), and (b) 4 months (August).

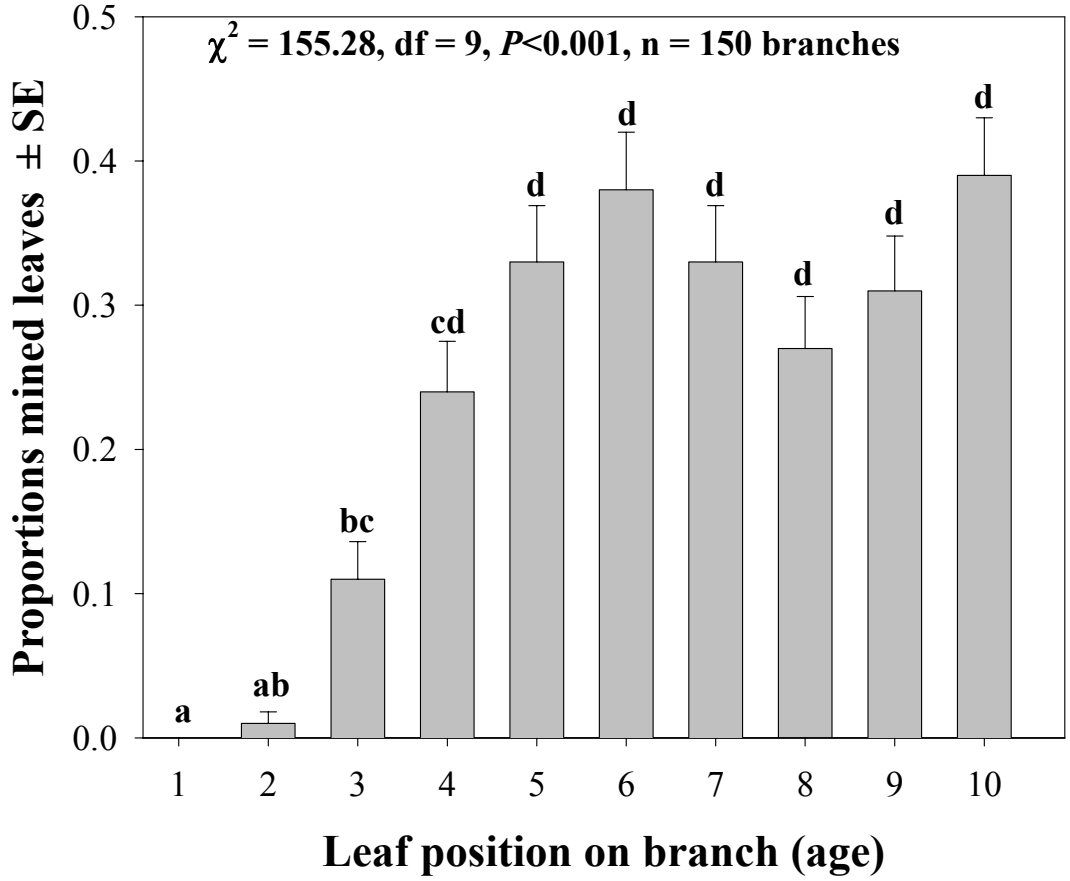

Fig. 2.2. Proportion mined leaves (± SE) in relation to leaf position on a branch, "*La Gloria*" coffee farm, Cacahoatán, Chiapas, Mexico. Leaf position on the branch was used as proxy for leaf age (leaves at position 1 are the youngest). Chi-square goodness of fit test and a Tukey-type multiple comparisons test were used for comparisons. Proportions do not differ significantly among leaf position on a branch at $\alpha = 0.05$ if the same letter appears above columns.

Leaf length, a proxy for leaf surface area, varied among plants from 9.47 to 13.97 cm (mean = 11.74 ± 0.09 cm). Proportion of mined leaves per plant at one ($r^2 = 0.0001$, $P = 0.96$) and four months ($r^2 = 0.005$, $P = 0.44$) were not significantly correlated with leaf size (n = 120) (fig. 2.4).

The penetrability indices of field-collected leaves varied between 51.5 and 125.2 g/mm^2 (mean = 90.4 ± 0.6 g/mm^2), and leaf specific weight between 0.39 and 1.12 g/cm^2 (mean = 0.68±0.01 g/cm^2). Mines occurred on coffee leaves with penetrability indices between 75.0 and 125.2 g/mm^2 (mean = 97.4 ± 1.6 g/mm^2), and specific weight between 0.39 and 1.12 g/cm^2 (mean = 0.65 ± 0.02 g/cm^2). Coffee leafminer larva size was not significantly correlated with leaf penetrability index ($r^2 = 0.003$, $P = 0.463$) or leaf specific weight ($r^2 = 0.002$, $P = 0.06$) (fig. 2.5).

Coffee leafminer oviposition preference in relation to leaf quality. Coffee leafminer females did not discriminate among leaf ages (position on branch) 1 to 6 in laboratory experiments ($P = 0.99$) (table 2.1), laying 2.2±0.5 eggs per leaf independently of leaf age. In contrast, they discriminated among leaf ages in field experiments, laying more eggs on the second and third pairs relative to the first pair, and more eggs on the third relative to the second pair of leaves ($P = 0.02$) (fig. 2.6).

Ovipositing coffee leafminer females did not discriminate in the laboratory among leaves with low, medium, or high nitrogen levels ($P = 0.92$) (1.8 ± 0.3 eggs per leaf), or between mined and mine-free leaves (3.4 ± 0.7 eggs per leaf) ($P = 0.35$) (table 2.1). However, they laid more eggs on large (1.6 ± 0.3 eggs/cm^2) versus small (0.4 ± 0.2 eggs/cm^2) and medium (0.9 ± 0.3 eggs/cm^2) sized leaves, while they did not discriminate between small and medium sized leaves ($\chi^2 = 7.89$, $P < 0.02$) (table 2.1).

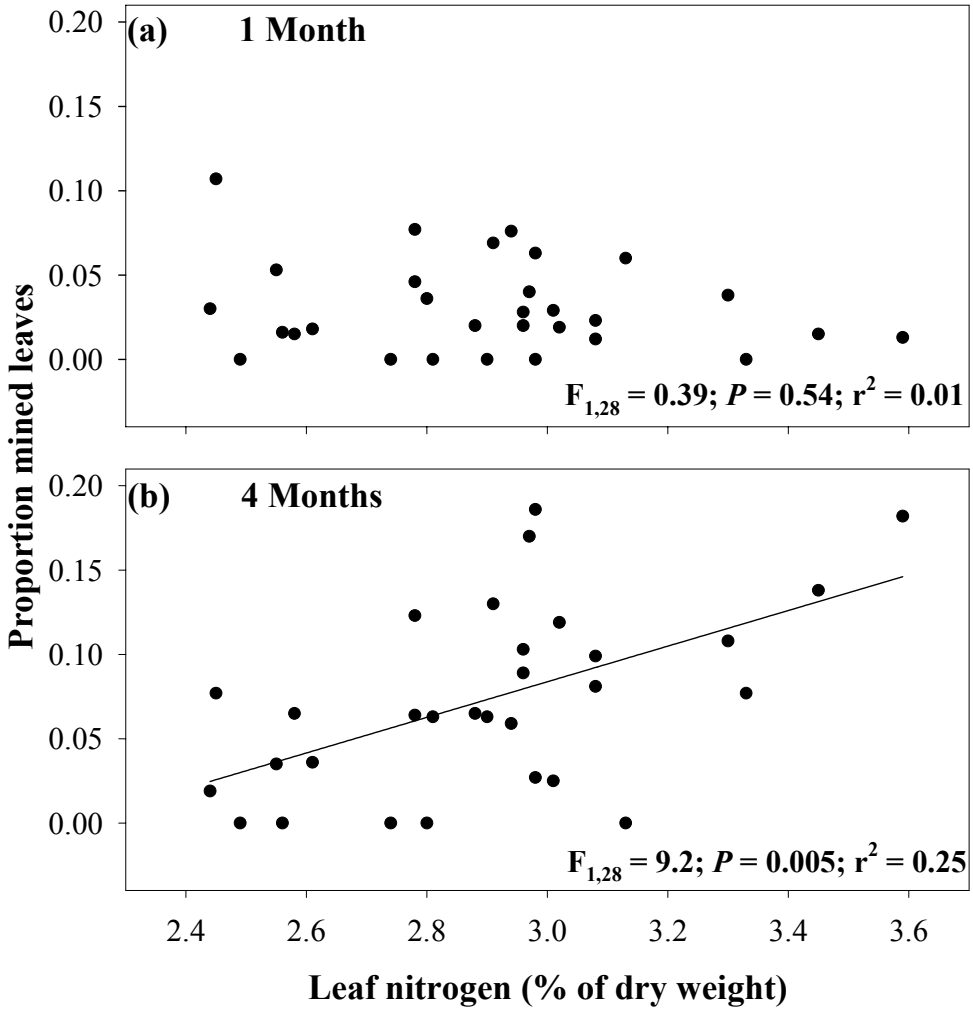

Fig. 2.3. Relationship between proportion mined leaves per coffee plant and leaf nitrogen content at (a) one (May, 2005), and (b) four months (August) of observation on leaves that originally were mine-free. Arcsine- √× transformed proportions of mined leaves were used for analyses, and untransformed values are shown in both (a) and (b).

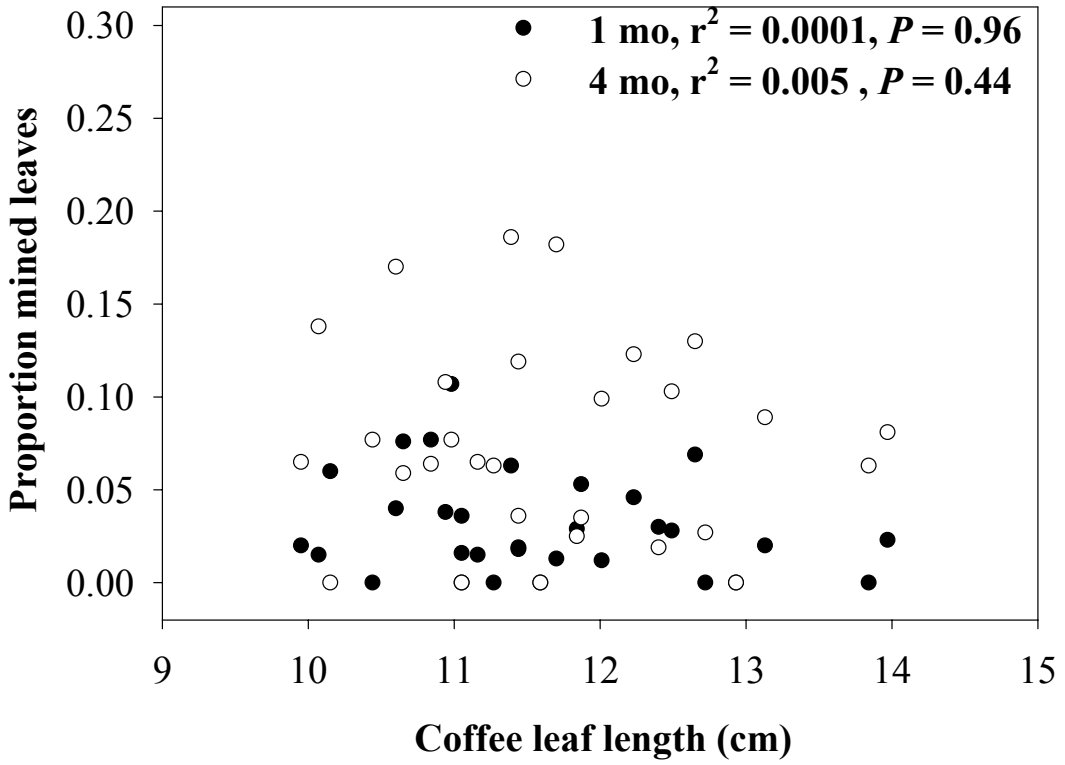

Fig. 2.4. Relationship between proportion mined leaves per coffee plant and coffee leaf length at one (May, 2005), and four months (August) of observation on leaves that originally were mine-free. Arcsine-√× transformed proportions of mined leaves were used for analyses, and untransformed values are shown in the graph.

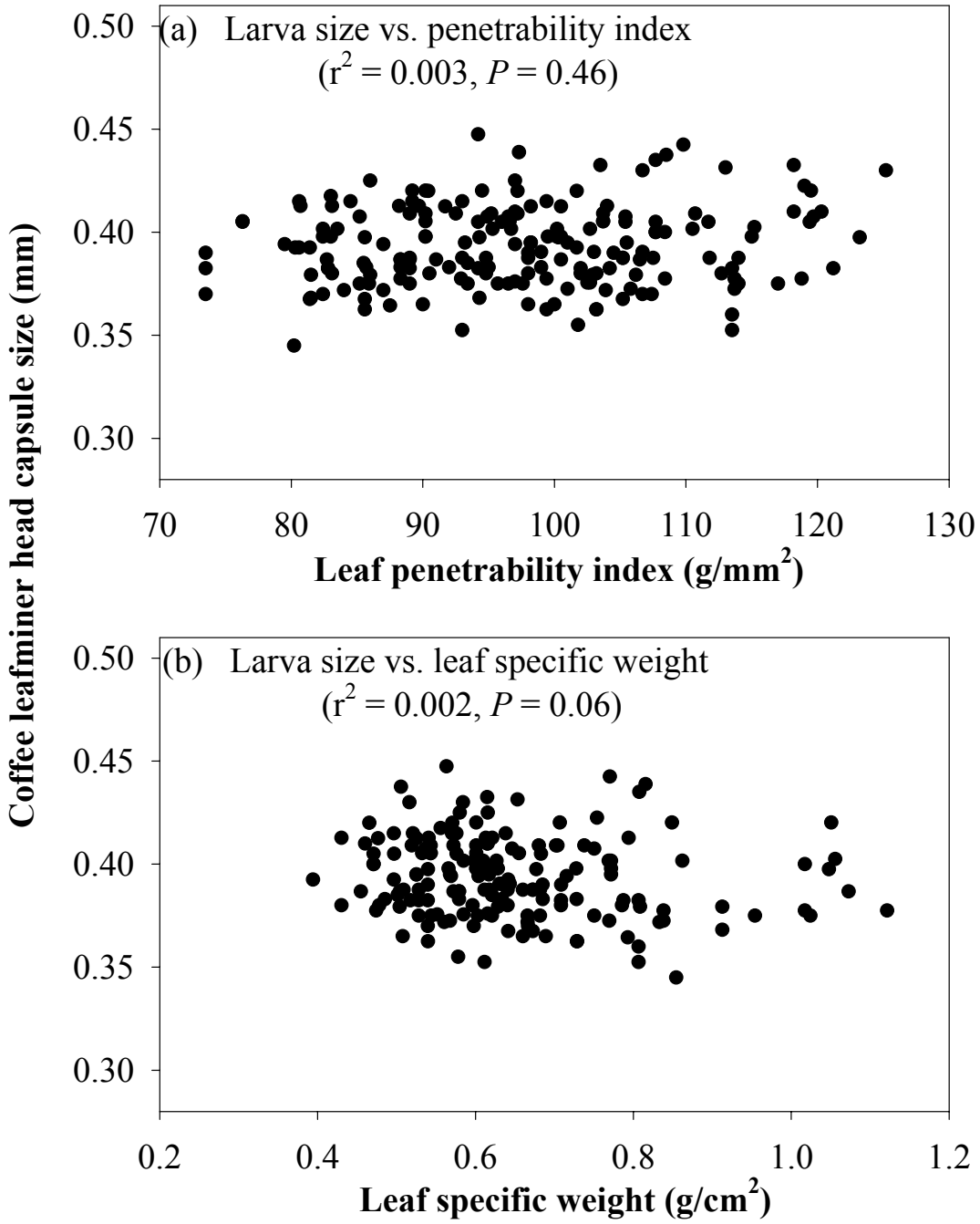

Fig. 2.5. Relationship between coffee leafminer last-instar larval head width and (a) coffee leaf penetrability index (g/mm^2) or (b) coffee leaf specific weight (g/cm^2) at "*La Gloria*" coffee farm, Cacahoatán, Chiapas, Mexico.

Table 2.1. Ovipositional discrimination by coffee leafminer females for leaves of different ages, nitrogen contents, or sizes, or for mined versus mine-free leaves. Statistics correspond to the Scheirer-Ray-Hare extension of the Kruskal-Wallis test.

Leaf quality parameter	**H**	**df**	***P***
Leaf age (position on a branch)	0.35	5	0.99
Leaf position in arena	0.46	5	0.99
Leaf nitrogen content	0.16	2	0.92
Leaf position in arena	6.00	5	0.31
Mined/mine-free leaf	0.87	1	0.35
Leaf position in arena	4.43	5	0.49
Leaf size	7.44	2	0.02
Leaf position in arena	2.71	5	0.75

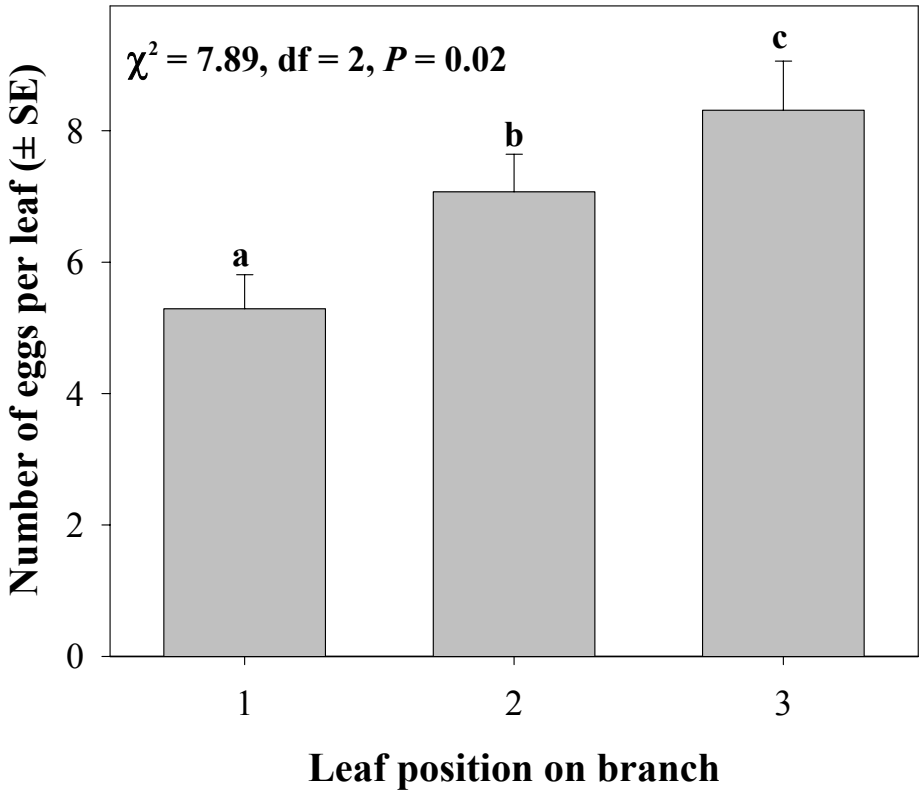

Fig. 2.6. Coffee leafminer oviposition (mean eggs laid/leaf ± SE) in relation to leaf position (age) on a branch on coffee plants in "*La Gloria*" coffee farm, Cacahoatán, Chiapas, Mexico. Data from four trials conducted on separate dates were pooled for analysis (χ^2 date = 6.84, df = 3, P = 0.08). Chi-square goodness of fit test and Tukey-type multiple comparisons were used for analyses. Means do not differ significantly among leaf position on branch at $\alpha = 0.05$ if the same letter appears above columns.

Coffee leafminer survival, size, and development time in relation to leaf nitrogen content and penetrability. Leaf nitrogen content. Adult males reared on leaves with low, medium, or high nitrogen levels did not differ significantly in size (P = 0.31) (fig. 2.7). In contrast, females reared on coffee leaves with medium levels of nitrogen were larger in relation to those reared on leaves with low or high nitrogen levels, but there was no significant difference between sizes of females reared on leaves

with low or high nitrogen levels ($P = 0.008$) (fig. 2.7). Coffee leafminers reared on leaves with moderate nitrogen content had a slightly shorter (<1 day) egg to pupa development time relative to those reared on leaves with low or high nitrogen content ($P < 0.001$) (fig. 2.8). Coffee leafminer survivorship from egg to larva (~0.95) ($F_{2,14} = 0.10$, $P = 0.91$), larva to pupa (~0.59) ($F_{2, 14} = 0.44$, $P = 0.66$), and pupa to adult (~0.55) ($F_{2, 14} = 0.31$, $P = 0.74$) did not differ significantly among leaf nitrogen levels.

Leaf penetrability index. Coffee leafminer larva size increased with increasing leaf penetrability index ($r^2 = 0.21$; $P < 0.001$) (fig. 2.9). Leaf penetrability weakly, though significantly, influenced egg to larva survivorship ($r^2 = 0.08$; $P < 0.001$) (fig. 2.10a), but did not affect larva to pupa survivorship ($r^2 = 0.03$; $P = 0.24$) (fig. 2.10b).

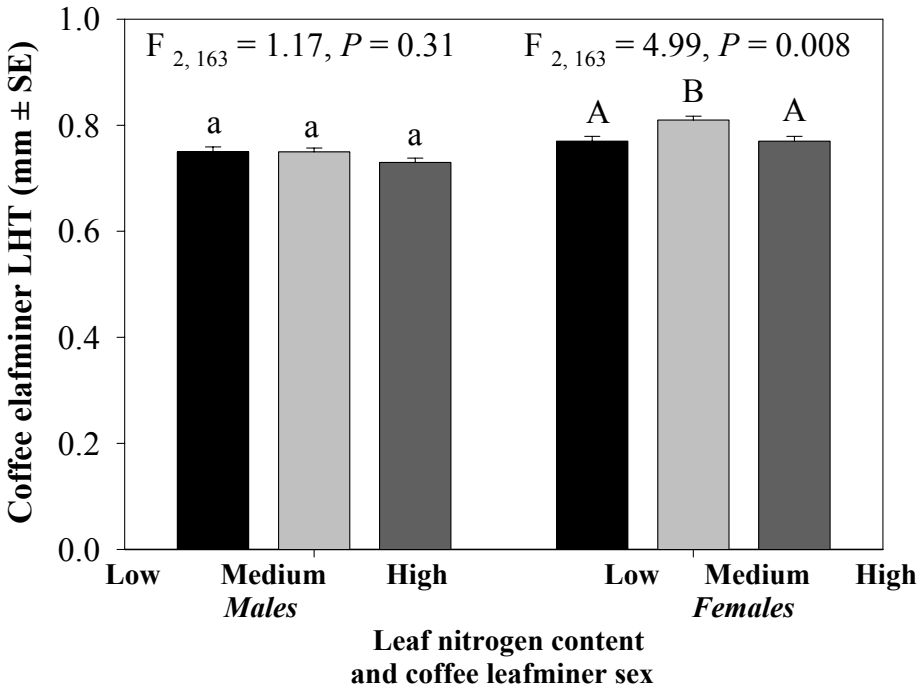

Fig. 2.7. Male and female coffee leafminer adult size based on mean length of hind tibia (LHT± SE) in relation to leaf nitrogen content (low = 2.53±0.04, medium = 2.90±0.01, high = 3.40±0.07). Means do not differ significantly among leaf nitrogen levels at $\alpha = 0.05$ if the same upper- or lower-case letter appears above columns. ANOVA and Tukey's HSD procedure were used to compare means among treatments within coffee leafminer sex.

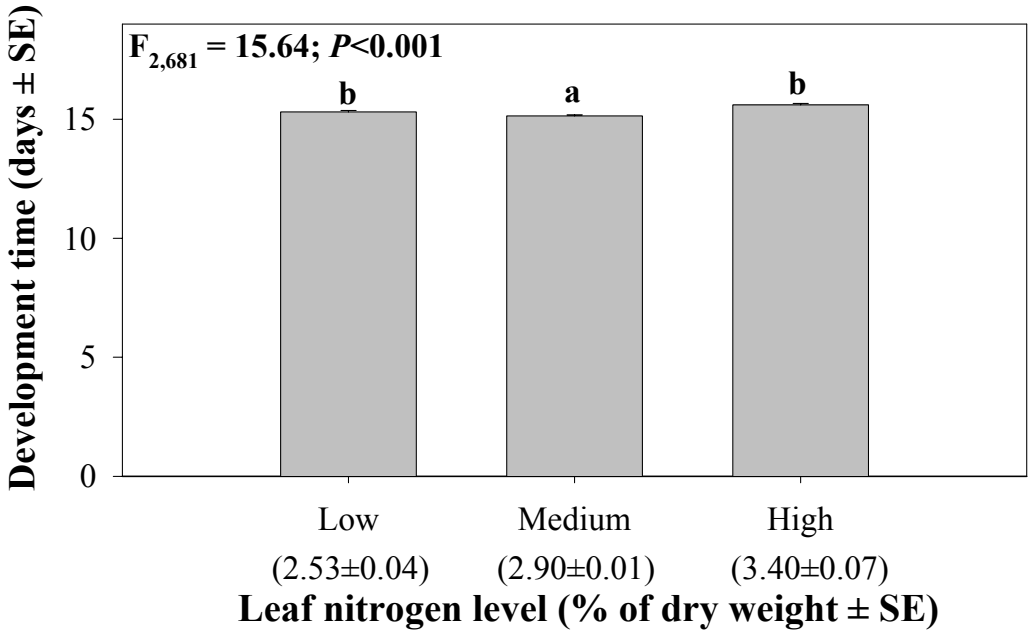

Fig. 2.8. Coffee leafminer mean development time (mean days ± SE) from egg to pupa on coffee leaves with one of three leaf nitrogen levels. Means do not differ significantly among treatments at $\alpha = 0.05$ if the same letter appears above columns. ANOVA and Tukey's HSD procedure were used to compare means among treatments.

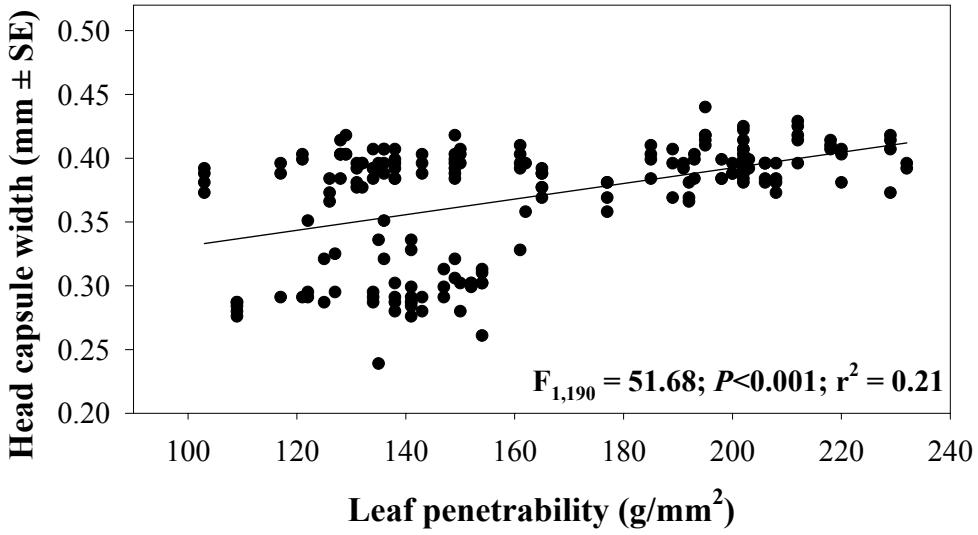

Fig. 2.9. Relationship between last-instar coffee leafminer larva size (mm ± SE), based on head capsule width, and leaf penetrability index (g/mm^2).

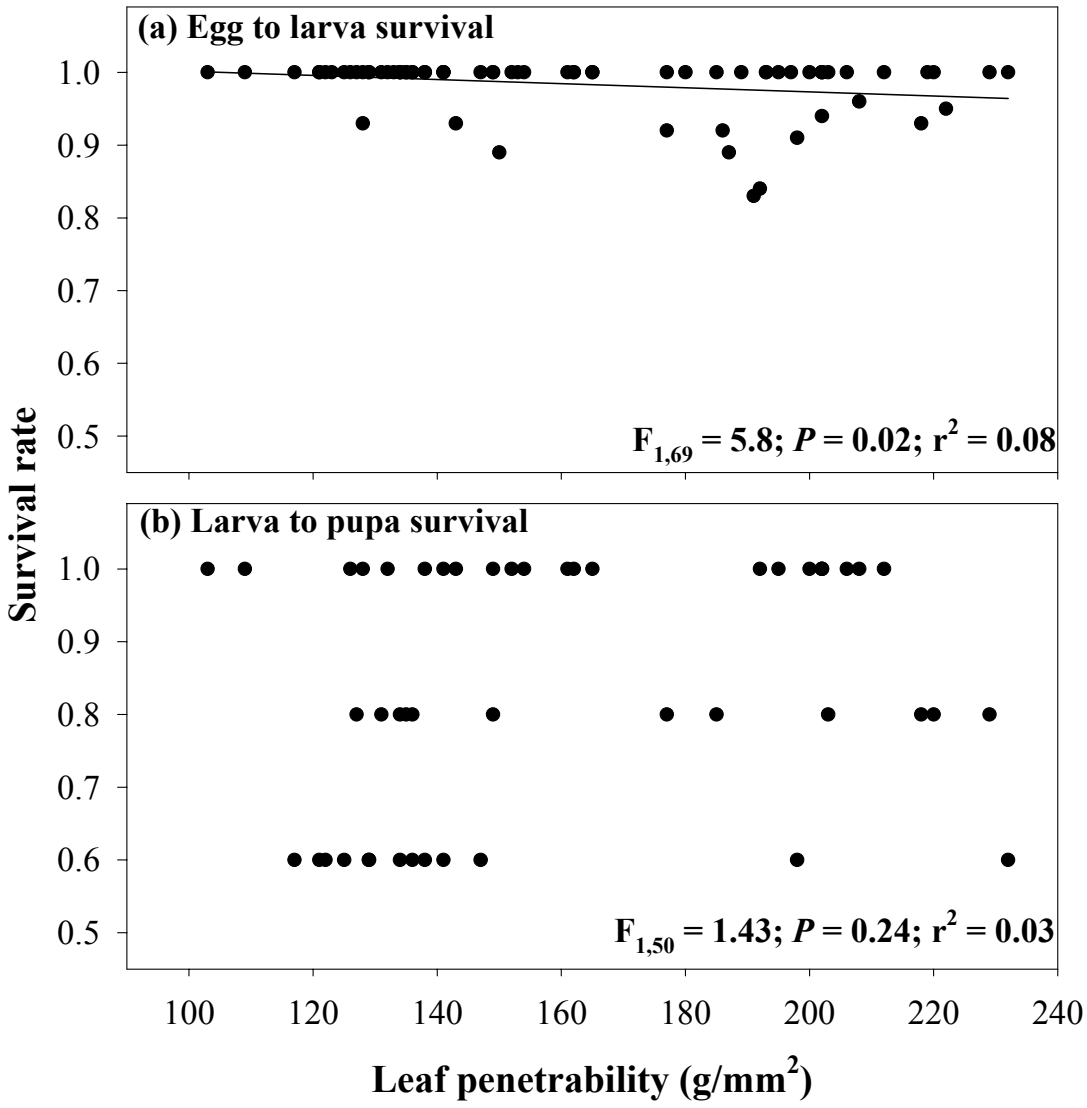

Fig. 2.10. Relationship between coffee leaf penetrability index (g/mm^2) and coffee leafminer (a) egg to larva, and (b) larva to pupa survivorship.

Discussion

Coffee plants were highly variable in various physical (leaf size, penetrability, specific weight) and chemical (leaf nitrogen content) properties evaluated in this study,

as well as in levels of coffee leafminer incidence, and this allowed testing some predictions of the optimal oviposition behavior hypothesis (Jaenike, 1978). Per previous studies on other herbivores and hosts plants, coffee leafminer was expected to occur more frequently in young and undamaged leaves, and in plants with large leaves, and with leaves with high leaf nitrogen content and low penetrability indices (Pfeiffer & Burts, 1983; Craig *et al*., 1989; Price, 1991; Ateyyat & Mustafa, 2001; Heisswolf *et al*., 2005). Also, higher coffee leafminer survival rates and larger adults were expected in leaves with high nitrogen content, high specific weight, and low leaf penetrability indices (Stamp & Bowers, 1990; Leather, 1994; Wheeler & Center, 1996; Awmack & Leather, 2002). The results of the present study were partially consistent with such expectations. Field surveys showed a greater proportion of mined leaves in plants with high leaf nitrogen content, though older leaves had lower nitrogen content, and mines occurred more frequently on older versus younger leaves within plants. Laboratory experiments showed that ovipositing females discriminated among leaf sizes, but not among leaf nitrogen contents or ages, nor between presence and absence of coffee leafminer mines. However, in field experiments coffee leafminer oviposited more frequently on older versus younger leaves. High predation levels on coffee leafminer observed in the field in the study area (see chapter III) may explain in part the discrepancy between laboratory and field observations, because it is possible that leafminers in older leaves were under a higher risk of predation, but this needs to be tested.

Leaf quality significantly affected coffee leafminer larval survival rate, growth, and development time, though not as expected. Coffee leafminers reared on leaves with medium nitrogen level had a slightly shorter development time and were larger relative to those reared on leaves with low or high nitrogen levels; however, the biological significance, if any, of the difference in development time is unclear because it was smaller than one day. And, coffee leafminers reared on leaves with high penetrability indices were larger than those reared on leaves with lower indices, but they had slightly lower egg to larva survivorship.

Many studies suggest that leaf nitrogen content and fertilization regimes affect herbivore populations in both natural and agricultural settings (Pfeiffer & Burts, 1983; Sipura & Tahvanainen, 2000; Ateyyat & Mustafa, 2001; Herms, 2002; Walde, 1995). For example, Ateyyat and Mustafa (2001) reported an increase in citrus leafminer population density, and a reduction in parasitism rates, on lemon trees with high levels of fertilization relative to control trees. Castillo *et al*. (1997) noted that fertilization was one of the most important cultural practices for coffee, and suggested that poor fertilizer management was related to greater incidences of pests and diseases, and to yield reduction. Thus, Castillo *et al*. (1997) recommended fertilizer management as a management tactic for coffee leafminer, though did not present data supporting this recommendation. In the present study, the leaf nitrogen content of the coffee plants was within the optimum range cited by Monge (1999), so the conclusions herein are limited to this condition. Field surveys showed a significant correlation between leaf nitrogen content and incidence of coffee leafminer, as predicted by the optimal oviposition

behavior hypothesis. However, coffee leafminer females in the laboratory did not discriminate for oviposition among leaves with different nitrogen contents. In addition, coffee leafminers reared on leaves from plants with moderate nitrogen content had higher survival rates and were larger than those reared on leaves with low or high nitrogen content. These data appear to follow the quadratic model (De Bruyn *et al*., 2002), which predicts that herbivores should have poorer performance at low and high nutrient levels relative to intermediate levels.

Coffee leafminers occurred in the field only in leaves with leaf penetrability indices between 73.5 and 125.2 g/mm^2, though leaves with lower indices were available. However, leaf penetrability in this study was measured when leafminer larvae emerged to pupate, so it is possible that penetrability was different when larvae were allocated at the egg stage to a leaf. Studies on other lepidopteran leafminers species suggested that leaf hardness (≈ leaf penetrability, Moran & Buchan, 1975), and leaf toughness (= leaf specific weight, Foss & Rieske, 2003) affect mine initiation by first-instar larvae, and make food acquisition more difficult by impeding food assimilation, so thereby affect survival, growth, weight gain, and performance of successive larval instars (Stamp & Bowers, 1990; Calvo & Molina, 2004). Coffee leafminer females do not insert their eggs in the leaf tissue, and larvae only feed on palisade parenchyma cells (Ramiro *et al*., 2004). Thus, first instar larvae must penetrate the leaf tissue to initiate mines, so high mortality and smaller leafminers were expected on leaves with high penetrability indices. Contrary to expectations, coffee leafminer survival rate from larva to pupa was not significantly affected by leaf penetrability indices, and larva size was positively

correlated with leaf penetrability indices. Most coffee leafminer larvae succeeded in penetrating the coffee leaf epidermis and initiating mines (~98%) independently of leaf penetrability, though survivorship from egg to larva was weakly correlated negatively with penetrability. Thus, as suggested for other Lyonetiidae leafminers (Kagata & Ohgushi, 2001), it is likely that under field conditions larval mortality resulted from chemical plant defenses or natural enemies, subsequent to mine initiation, rather than from leaf physical characteristics, such as penetrability or toughness.

Leaf quality usually changes with leaf age (Stamp & Bowers, 1990; Forister, 2005). For example, Guerreiro-Filho (2006) found a higher concentration of phenol compounds in young coffee leaves relative to old leaves, and suggested that secondary metabolites were transferred from old to young leaves for greater protection against herbivory. Moreover, Guerreiro-Filho (2006) noted that caffeine levels increased during coffee leaf expansion, and caffeine was higher in young versus old leaves. In the present study, the leaf nitrogen content of older leaves was significantly lower than that of younger leaves; also, previous studies suggested higher leaf penetrability indices in old versus young leaves (Guerreiro-Filho, 2006). Thus, ovipositional preference for young leaves was expected, but coffee leafminer females in the laboratory did not discriminate among coffee leaf ages to lay eggs, though in the field, young leaves less frequently hosted coffee leafminer mines. The difference between laboratory and field preference for young leaves may have been due to differences in the spatial distribution of leaves. In the field experiment, coffee leafminers chose among leaves at different position along

a branch axis, while in the laboratory experiments they chose among leaves placed in a circle in an arena.

Similar to the results of the present study, previous studies showed that older leaves were more susceptible to coffee leafminer damage than younger leaves (Walker & Quintana, 1969; Nantes & Parra, 1977; Guerreiro-Filho, 2006). While the frequency of mines on old versus young leaves could be due to greater exposure time, in the present study the influence of exposure time was minimized by focusing the field survey on leaves with fresh damage and initiating field observations with undamaged leaves, and conducting experiments with laboratory reared coffee leafminers. In the laboratory, ovipositing coffee leafminer females discriminated among coffee leaf sizes. More eggs per cm^2 were laid on large leaves than on small leaves of similar age. However, field surveys did not show a significant relationship between plant mean leaf size and proportion of mined leaves. Although coffee leafminer offspring performance was not evaluated in relation to leaf size, studies on other insects (Heisswolf *et al.,* 2005) support the "plant vigor hypothesis" (Price, 1991), which predicts that females should prefer large and vigorously growing host plants for oviposition, and their larvae should perform better on those plants. Coffee plants with large leaves have high coffee bean yields (Blanco *et al*., 2003), so leaf size is likely associated with plant quality in coffee plants.

In summary, laboratory experiments showed that leaf nitrogen content, age, and penetrability index affected coffee leafminer survival, development time, and offspring size, but ovipositing coffee leafminer females did not discriminate generally in favor of leaves that maximized offspring performance. Also field observations on leaf nitrogen

content and penetrability indices did not generally coincide with those the results of laboratory experiments: coffee leafminer incidence was higher in the field on leaves with higher nitrogen content, while in the laboratory larger leafminers were obtained from leaves with medium nitrogen levels; coffee leafminer incidence was not correlated with leaf penetrability indices in field, but in the laboratory egg to larva survivorship was negatively (though weakly) and larva size was positively correlated with leaf penetrability indices. The results of this study were only partially predicted by the optimal oviposition behavior hypothesis (Jaenike, 1978; Thompson, 1988; Scheirs, 2002). However, other studies suggested that herbivore performance is determined not only by plant quality, but also by natural enemies, environmental characteristics, and larval competition (Valladares & Lawton, 1991; Kagata & Ohgushi, 2001; Moon & Stiling, 2006). It is likely that coffee leafminer egg distribution patterns in relation to host plant quality parameters differed between field and laboratory observations due to egg predation, which may exceed 90% in the field (see chapter III). The results of the present study together with others, suggest that investigating predictions of the *enemy-free space hypothesis* in relation to coffee leafminer is warranted. Studies carried out in coffee farms in the Soconusco region, Chiapas, and in other areas, demonstrated that coffee leafminer suffers high mortality rates due to natural enemies (Greenberg, *et al.,* 2000; see chapters III and IV), and weather factors (see chapter V), which may be important factors influencing coffee leafminer distribution and abundance.

CHAPTER III

IMPACT OF NATURAL ENEMIES ON COFFEE LEAFMINER *Leucoptera coffeella* (LEPIDOPTERA: LYONETIIDAE) POPULATION DYNAMICS

Introduction

The leaf-mining habit allows leafminers to mitigate the detrimental effects of the physical environment, some physical plant defenses, predators, and pathogens, though disadvantages, such as lower fecundity, smaller body size, and greater mortality due to leaf abscission and parasitism relative to externally feeding folivores have been documented (Connor & Taverner, 1997). Leafminers are relatively sessile, so are highly susceptible to chemical plant defenses and reduces their chances of escaping parasitism (Cornell & Hawkins, 1995). While examining leafminer mortality, Auerbach *et al.* (1995) identified parasitism of larvae, and in some cases of pupae, as important sources of mortality, and Auerbach (1991) found that leafminers apparently suffered little egg mortality due to natural enemies.

Coffee leafminer, *Leucoptera coffeella* (Guérin-Ménèville) (Lepidoptera: Lyonetiidae), is a nocturnally active microlepidopteran species whose larvae are known only from *Coffea* species (Mendoza, 1995; Ramiro *et al*., 2004). Previous studies addressing the effects of coffee plants on coffee leafminer distribution and abundance, showed that coffee leafminer incidence was positively correlated with leaf nitrogen content and age (= position on a branch) (chapter II). In addition, laboratory studies showed that coffee leafminer grew larger, developed faster, and survived better on

leaves with moderate nitrogen levels versus leaves with low or high nitrogen levels, and on tough versus soft leaves (Walker & Quintana, 1969; Nantes & Parra *et al*., 1977; Guerreiro-Filho, 2006; chapter II). However, coffee leafminer adult females, did not generally select such leaves for oviposition in laboratory experiments (chapter II). Under field conditions, natural enemies and weather variables may have stronger influences than host plant attributes on the distribution and abundance of coffee leafminer (Heisswolf *et al*., 2005); chapters II, V). Studies assessing the impacts of different mortality factors suggested that coffee leafminer population dynamics were strongly affected both by natural enemies (Flórez & Hernández, 1981; Gravena, 1983; Campos *et al*., 1989; Paliz & Mendoza, 1993), and environmental conditions prevalent at different elevations (e.g., de Souza, 2003; chapter V).

Prior studies documented a diverse suite of coffee leafminer natural enemies, including at least 26 genera and 30 species of larval parasitoids, all Eulophidae or Braconidae (Hymenoptera), though no egg or pupal parasitoids were documented (chapter IV). A compilation of predator species from previous studies yields at least six genera and 11 species of Vespidae, one morphospecies of Formicidae (Hymenoptera), and two of Chrysopidae (Neuroptera), all predatory on larvae; predators of pupae and eggs have not been reported (Enriquez *et al*., 1975; Parra *et al*., 1977; Tozatti & Gravena, 1988; Mendoza, 1995; Fragoso *et al*., 2002; Carvalho *et al*., 2002; Fernández & Bueno, 2002; Carvalho *et al*., 2005). However, most studies focused exclusively on coffee leafminer larval mortality (e.g., Parra *et al*., 1977; Villacorta, 1980; Aranda-Delgado, 1986; Reis *et al*., 2000). Moreover, preliminary studies conducted in coffee

farms in Chiapas, Mexico, showed that coffee leafminer eggs and pupae suffered high mortality rates due to predation (JRLF, unpubl. data).

Coffee leafminer is a potentially important pest of coffee in Mexico, but few studies have assessed the impact of natural enemies. Aranda-Delgado (1986) conducted the only work done in Mexico on coffee leafminer parasitoids, but its primary focus was on inventorying larval parasitoids. In addition, coffee leafminer in prior studies showed higher densities in a low versus high elevation farm (chapter V), but it was unclear whether such a difference was due to natural enemies or weather-related factors. Thus, further research on the effects of natural enemies is needed targeting all coffee leafminer developmental stages under varying weather conditions. Specifically, research quantifying the effects of natural enemies on the population dynamics of coffee leafminer under different weather conditions is needed. Data derived from such research are necessary for incorporation into evolving coffee IPM programs (Barrera, 2002; Segura *et al*., 2004). The present study thus sought to: 1) identify the natural enemy complex associated with coffee leafminer, and 2) contrast the effects of natural enemies on coffee leafminer population dynamics at two elevations and two rainfall levels, representing contrasting weather conditions in the Soconusco region of Chiapas, Mexico.

Materials and methods

Study area. Studies were conducted in three coffee farms representing two elevations in the municipality of Cacahoatán, Chiapas, Mexico: El Encanto (14^{o} 59’ 21’’

N; 92 º 09’ 55’’ W; 480 m) and La Gloria (15º 00’ 07’’ N; 92 º 09’ 24’’ W, 550 m), representing low elevation farms, and Alpujarras (15º 04’ 24’’ N; 92 º 10’ 11’’ W, 960 m) representing high elevation farms. The main coffee varieties in these farms were “Borbon,” “Typica,” and “Catuai,” but all studies involved Catuai plants. Insecticides were not used in any of the farms during the study period.

Natural enemy complex associated with coffee leafminer. Two hundred leaves with active coffee leafminer mines, and 100 coffee leafminer pupae were collected monthly at one low elevation (El Encanto) and the high elevation farm (Alpujarras) during the summers (May to July) of 2003 to 2005 to document the parasitoid community associated with coffee leafminer. In addition, sporadic collections were made in these and other coffee farms in the Soconusco region to recover as many parasitoid species as possible. Leaves with active mines or pupae were taken to the laboratory and examined under a microscope at 25× magnification (Carl Zeiss Inc., New York). All coffee leafminer eggs, larvae, and pupae recovered from leaves were incubated individually in 5 cm diam Petri dishes until adult parasitoids or adult leafminers emerged. Adult parasitoids were killed in 80% alcohol, and curated for subsequent identification. The specimens were identified to genus using the keys of Gibson *et al.* (1997), Burks (2003), and Wharton *et al.* (1997). Species within each genus were identified using specific keys (see chapter IV).

A preliminary survey of potential predators of coffee leafminer was conducted in mid-July 2005. Thirty coffee plants were randomly selected in a low elevation farm (La Gloria), and all predators found on each plant during a 10 min per plant observation

period were collected, including ants living inside coffee twigs or branches. Also, any arthropod observed preying on coffee leafminers during the parasitoid surveys described above was collected, though few collections were made in this manner because coffee leafminer eggs and pupae were infrequent, and because of a likely short interaction time between predators and coffee leafminer prey. Collected predators were killed in 80% alcohol, and curated for subsequent identification. The specimens thus collected, all ants (see *Results*), were identified to genus using the keys of Mackay & Mackay (2004).

Sentinel coffee leafminer eggs, larvae, and pupae were exposed on coffee plants to collect predators during the last week of July 2005 at a low elevation farm (La Gloria). Sentinel coffee leafminers were reared at El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, Mexico, on detached coffee leaves as described in Reis *et al.* (2000) and Chapter II. Sentinel eggs, larvae, and pupae were exposed on 10 randomly selected coffee plants by stapling three coffee leaves with mature mines (mined area >4 cm^2), three with coffee leafminer eggs (<24 h old), and three with pupae (<24 h old) to leaves on each plant. Together, at least 30 eggs, five larvae, and five pupae were thus exposed per plant. Plants with sentinels were in a single row, and separated from each other by at least five coffee plants. Diurnal (9:00-13:00 h) and nocturnal (20:00-0:00 h) observations were made to document predators preying on the coffee leafminer sentinels. Each coffee leaf with sentinels was observed for a period of 5 sec every 6 min in a rotating fashion. Almost 400 observations were made during a total observational period of 32 h, i.e. 16 h during the day and 16 h during the night. Predators feeding on sentinels were identified to genus in the field, or collected for subsequent

identification, and the frequencies of predation events were scored. A predation event was recorded when a predator was observed consuming/removing sentinel eggs, larvae, or pupae from coffee leaves. Sentinels were replaced as they were predated.

Effects of natural enemies on coffee leafminer population dynamics. The seasonal dynamics of coffee leafminer was assessed through monthly surveys at a low elevation farm (El Encanto) from June 2003 to July 2004. Ten coffee plants were randomly selected each month and the proportion of mined leaves per plant was assessed from nine branches per plant.

Key factor analysis (Varley *et al*., 1974; Bellows & van Driesche, 1999) was performed to elucidate the impact of natural enemies on coffee leafminer population dynamics. One hundred-and-eighty mined leaves and 100 coffee leafminer pupae were collected per month. Leaves were held in plastic bags, and examined later for coffee leafminer survivorship and mortality sources. The numbers of mines, and coffee leafminer eggs and larvae on each leaf were recorded. Coffee leafminer larval and pupal survival rates and mortality factors were assessed in the laboratory through dissection under a microscope at 25× magnification. Egg survival rate could not be assessed via field samples because most of the mortality at this stage is due to predation, and predators did not leave visible egg remains as evidence of predation (JRLF, pers. observ.).

Previous studies (Aranda-Delgado, 1986; JRLF, unpubl. data) showed that larval and pupal coffee leafminer mortality could be assigned to one of three factors. 1) Death by parasitoid: indicated by the presence of immature parasitoids (larvae, pupae, or their

remains) inside coffee leafminer mines or pupae, parasitoid exit holes, or by presence of black, ovipositor-caused scars on coffee leafminer larvae. 2) Death by predator: indicated by torn mines in which the number of larvae within the mine did not correspond to the number of eggshells at the base of the mine, or by torn or empty coffee leafminer pupal remains in the absence of evidence of parasitoid activity. 3) Death by factors other than parasitoids or predators (hereafter FOPP): coffee leafminer larval or pupal mortality was assigned to FOPP when mortality could not be unambiguously assigned to either death by parasitoid or predator. Destructive host feeding by some parasitoids was likely common, e.g., *Cirrospilus* sp. and *Pnigalio* sp. (Urbaneja *et al*., 2001), but could not be determined from field-collected material, so an unknown proportion of coffee leafminer mortality assigned to FOPP may have been due to parasitoids.

Regression analyses were conducted to uncover relationships between total mortality (*K*) and monthly coffee leafminer density per leaf (= number of larvae/leaf), and between individual mortality factors (*k*) and total mortality (*K*), following Varley *et al.* (1974). Monthly coffee leafminer density per leaf was calculated by multiplying the proportion of mined leaves per plant by the mean number of coffee leafminer larvae per leaf, which were assessed from the monthly surveys, as described above.

Contribution of natural enemies to coffee leafminer mortality at two elevations and rainfall levels. Life table analysis (Southwood, 1978; Bellows & van Driesche, 1999) was used to elucidate the impact of natural enemies on coffee leafminer survival at one low elevation farm (La Gloria) and the high elevation farm, during

periods of low and high rainfall. Coffee leafminer survival and mortality during the larval stage were assessed by following coffee leafminer cohorts established on 10 randomly selected coffee plants. One branch without mined leaves was selected per plant. The apical three pairs of leaves on each branch were enclosed in a 30 cm nylon mesh sleeve. Two pairs of coffee leafminer adults (24 h-old) were enclosed in each sleeve for 48 h. After this time, the number of eggs laid was recorded and adults removed. Sleeves were left on the branch for 1 wk to exclude egg predators, and allow larvae to initiate mines, and removed afterward to expose coffee leafminer larvae to natural enemies until they completed their development (~1 wk under local field conditions). At the same time, 10 coffee leaves with coffee leafminer eggs or pupae reared in the laboratory were exposed to natural enemies, by stapling them to coffee leaves, on each of the 10 selected plants. At least 50 eggs (5-15/leaf), 10 pupae (1 or 2/leaf), and 40 larvae (5-38/cohort) were exposed per plant. All eggs, larvae, and pupae were exposed to natural enemies for 1 wk. After 1 wk, leaves with eggs, mines, or pupae were taken to the laboratory and examined under a microscope at 25× to assess stage-specific survivorship rate, and mortality rates due to parasitoids, predators, and FOPP. Two life tables were thus constructed at each elevation, the first beginning on April 27 (2005), during a period of low rainfall (total rainfall <100 mm), and the second beginning on June 2 (2005), during a period of high rainfall (>400 mm). The numbers of coffee leafminers surviving and dying in each stage per each of the 10 cohorts were combined for life table analyses. Real mortality, the ratio of those dying in a given stage to the number initially entering the first stage in a life table (Bellows & van Driesche,

1999), and indispensable mortality, that which would not be replaced in the population by the subsequent action of other mortality factors if the factor under consideration were removed (Bellows & van Driesche, 1999), were used to compare the impacts of predation, parasitism, and FOPP within each elevation and rainfall level. Apparent mortality, the ratio of the number dying in a stage to the number entering that stage (Bellows & van Driesche, 1999), was used to make comparisons among localities and rainfall levels within coffee leafminer stages. Mutual independence among the distribution of mortality rates due to specific factors (death by predation or FOPP of eggs or pupae, or by predation, parasitism, or FOPP of larvae), rainfall levels, and elevations was tested using a 3-dimensional contingency table *G*-test, followed by partial independence tests for the distribution of mortality rates and elevations or rainfall levels (Zar, 1996). Specifically, the hypothesis that mortality rate distributions were independent of elevation and rainfall level was tested using frequency data from mortality factors acting on eggs, larvae, and pupae from the four life tables that were assembled, i.e. low elevation+low rainfall, low elevation+ high rainfall, high elevation+low rainfall, and high elevation+high rainfall; independence between mortality rate distributions and rainfall levels was tested by comparing low versus high rainfall level life tables within each elevation, and independence between mortality rate distributions and elevations was tested by comparing low versus high elevation life tables within each rainfall level. Comparisons of mortality rates due to parasitoids, predators, or FOPP within each of the four life tables were made via *G*-tests, and Tukey-type tests, as warranted (Zar, 1996).

Results

Natural enemy complex associated with coffee leafminer. Parasitoids. A total of 791 parasitoid specimens were collected during the present study, consisting of at least 20 morphospecies of Eulophidae and two of Braconidae (table 3.1). The parasitoid genera recovered most frequently were *Neochrysocharis* (five morphospecies, 284 specimens), followed by *Stiropius* [one species, *S. letifer* (Mann), 113 specimens], *Closterocerus* (three morphospecies, 100 specimens), *Pnigalio* (three morphospecies, 98 specimens), and *Zagrammosoma* (two morphospecies, 64 specimens). The remaining five genera were each represented by fewer than 50 specimens (table 3.1). The five most abundant genera, as listed above, included >83% of the specimens recovered. Though ~50% of the total specimens were collected at one of the low elevation farms (El Encanto), and ~10% at the high elevation farm (table 3.1), almost the same numbers of morphospecies were recovered at both elevations, 18 and 19 respectively; *Allobracon* sp. (Braconidae) was recovered only in the low elevation farm.

All parasitoid specimens recovered from coffee leafminer were larval parasitoids, and most were recovered from mines (~78%, 18 morphospecies), and some emerged from coffee leafminer cocoons (~22%, 4 morphospecies). Egg or pupa parasitoids were not recovered. Primary parasitoids (18 morphospecies) represented ~82% of the species recovered, secondary parasitoids were not recovered, and likely facultative secondary parasitoids (4 morphospecies) were infrequent.

Predators. Thirteen ant (Formicidae) morphospecies were recovered on the 30 coffee plants that were sampled. The most abundant ant species were *Pseudomyrmex* sp.

1 (on 60% of the plants), *Leptothorax* sp. (37%), and *Brachymyrmex* sp. (30%). The remaining 10 ant species were present on <15% of the plants (fig. 3.1). *Brachymyrmex* sp. was the only ant species collected on coffee plants that was not observed preying on sentinel coffee leafminers during this study (see below).

Seventy-eight predation events on sentinel eggs, larvae, and pupae were observed during 16 h of diurnal observations, and two events during 16 h of nocturnal observations (fig. 3.2). Most predation events observed during the day involved ants (77/78), except one involving a spider (Salticidae). One predation event observed during the night involved an ant, *Cephalotes* sp., and the other a cockroach nymph (Blattaria). Predation events most frequently involved *Camponotus* sp. 1. (~40% of events), *Pseudomyrmex* sp. 3 (~23%), *Azteca* sp. (~11%), and *Pseudomyrmex* sp. 1 (~10%). The remaining species together accounted for ~16% of the total predation events. Ten predation events were observed on coffee leafminer eggs by four ant species (*Azteca* sp., *Crematogaster* sp., *Pseudomyrmex* sp. 2, and *Cephalotes* sp.), 13 events on larvae by four ant species (*Pseudomyrmex* sp. 1, sp. 2, sp. 3, and *Camponotus* sp. 1), and 57 events on pupae by six ant species (*Pseudomyrmex* sp. 1, sp. 2, sp. 3, *Leptothorax* sp., *Azteca* sp., *Camponotus* sp. 1), one spider species (Salticidae), and one cockroach species (fig. 3.2). Overall, sixteen predator species were observed preying on coffee leafminer, including 13 Formicidae, one Vespidae, one Blattaria, and one Salticidae, and pupa was the coffee leafminer stage most used as a prey (table 3.2).

Table 3.1. Parasitoid species and numbers of specimens recovered from coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) from coffee farms in the Soconusco region of Chiapas, Mexico.

		No. of specimens (%) by locality			
Genus/species	**Habit[2]**	**Alpujarras** N 15° 04' 24'' W 92° 10' 11'' 960m	**El Encanto** N 14° 59' 21'' W 92° 09' 55'' 480m	**Other[1]**	**Total**
Eulophidae					
Cirrospilus spp.[3]	L, FS, Ec	2 (2.5)	4 (1.0)	15 (4.7)	21 (2.7)
Closterocerus spp.[3]	L, PP, En	26 (32.9)	11 (2.8)	20 (6.3)	57 (7.2)
C. cinctipennis Ashmead	L, FS, En	1 (1.3)	35 (8.9)	7 (2.2)	43 (5.4)
Elachertus spp.[3]	L, PP, Ec	2 (2.5)	16 (4.1)	29 (9.1)	47 (5.9)
Horismenus spp.[3]	L, PP, Ec	3 (3.8)	8 (2.0)	2 (0.6)	13 (1.6)
Miotropis sp.	L, PP, Ec	3 (3.8)	3 (0.8)	0 (0)	6 (0.8)
Neochrysocharis spp.[3]	L, PP, En	10 (12.7)	98 (24.9)	87 (27.3)	195 (24.7)
N. arvensis Gram.	L, PP, En	1 (1.3)	13 (3.3)	14 (4.4)	28 (3.5)
N. chalybea Hansson	L, PP, En	3 (3.8)	7 (1.8)	13 (4.1)	23 (2.9)
N. formosa (Westwood)	L, FS, En	1 (1.3)	27 (6.9)	10 (3.1)	38 (4.8)
Pnigalio spp.[3]	L, PP, Ec	6 (7.6)	28 (7.1)	39 (12.2)	73 (9.2)
P. sarasolai De Santis	L, PP, Ec	3 (3.8)	17 (4.3)	5 (1.6)	25 (3.2)
Zagrammosoma lineaticeps (Girault)	L, PP, Ec	0 (0)	0 (0)	1 (0.3)	1 (0.1)
Z. multilineatum (Ashmead)	L, PP, Ec	1 (1.3)	30 (7.6)	32 (10.0)	63 (8.0)
Braconidae					
Allobracon sp.	L, PP, En	0 (0)	45 (11.5)	0 (0)	45 (5.7)
Stiropius letifer (Mann)	L, PP, En	17 (21.5)	51 (13.0)	45 (14.1)	113 (14.3)
TOTAL (% specimens)		79 (~10%)	393 (49.7%)	319 (40.3%)	791

[1] Localities where collections were unsystematic.

[2] L= larval parasitoid; PP= primary parasitoid; FS= facultative secondary parasitoid; Ec= ectoparasitoid; En= endoparasitoid.

[3] Includes at least 2 unidentified species.

Table 3.2. Predator species observed preying on coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) eggs, larvae, or pupae during 16h each of diurnal and nocturnal observation in a low elevation coffee farm (La Gloria , 15° 00' 07'' N; 92 ° 09' 24'' W, 550 m), Cacahoatán, Chiapas, Mexico.

	Coffee leafminer stage used as prey		
Genus/species	**Egg**	**Larva**	**Pupa**
Vespidae Unidentified		X	
Formicidae			
Azteca sp.	X		X
Camponotus sp. 1		X	X
Camponotus sp. 2	X		
Camponotus sp. 3	X		
Cephalotes sp.	X		
Crematogaster sp.	X		
Leptothorax sp.			X
Monomyrmex sp.			X
Pseudomyrmex sp. 1		X	X
Pseudomyrmex sp. 2		X	X
Pseudomyrmex sp. 3		X	X
Solenopsis sp. 1			X
Solenopsis sp. 2			X
Blattaria Unidentified			X
Salticidae (Arachnida) Unidentified			X

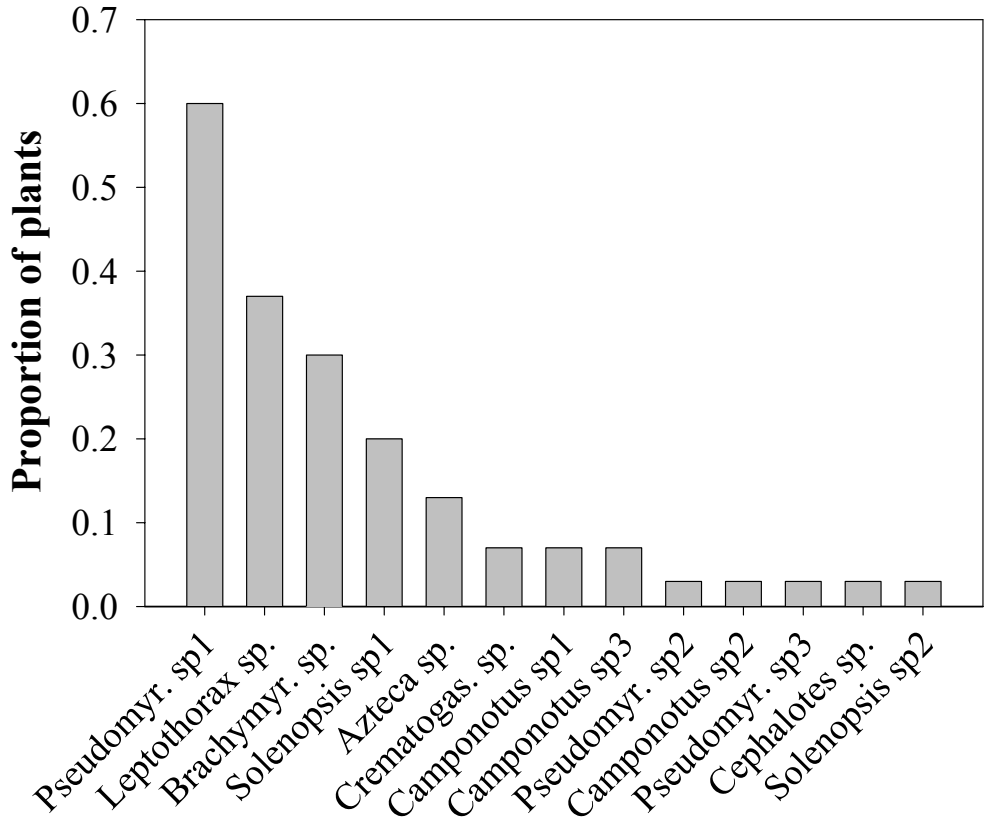

Fig. 3.1. Predatory species collected in a 10 min period on each of 30 coffee plants in a low elevation farm (La Gloria, 550 m), Cacahoatán, Chiapas, Mexico. Bars show the proportion of coffee plants on which each species was collected.

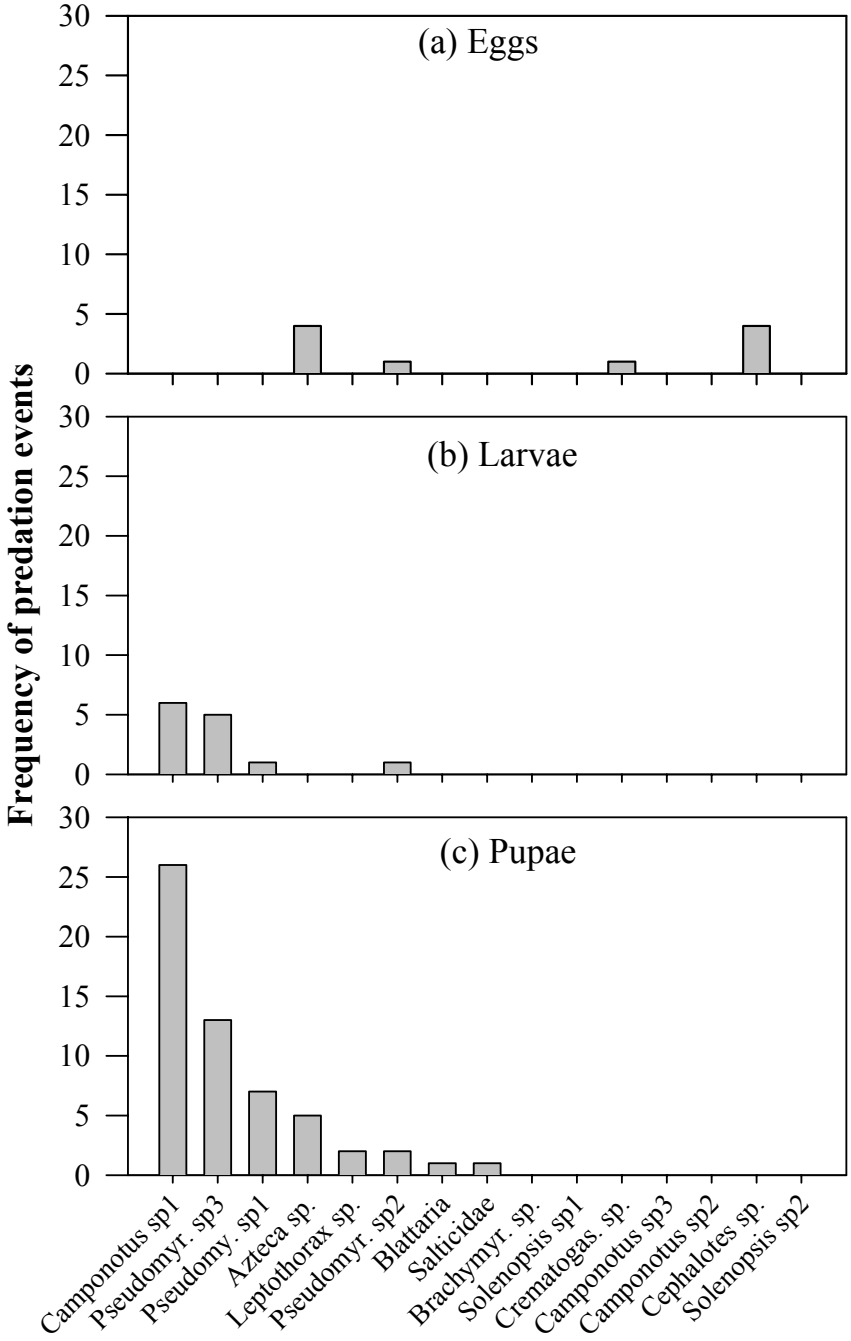

Fig. 3.2. Predation events by individual predator species on coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) sentinel eggs (a), larvae (b), and pupae (c). Predation frequencies are from 384 point (5 sec) observations during 16 h of diurnal and 16 h of nocturnal observations (July, 2005) in a low elevation coffee farm (La Gloria, Cacahoatán, Chiapas, Mexico, 550 m).

Effects of natural enemies on coffee leafminer population dynamics. Coffee leafminer was present throughout the study period, June 2003 to July 2004 (fig. 3.3a). The coffee leafminer monthly density (= larvae/leaf) varied between 0.10 and 1.41 larvae/leaf with an average of 0.70±0.42 (fig. 3.3a). Coffee leafminer density was generally higher during the rainy relative to the dry (December 2003-March 2004) season, and was >0.90 larvae/leaf during most (May-October) of the 2003 rainy season, and was highest (~1.41 larvae/leaf) in June-July 2004. The lowest coffee leafminer density, <0.15, occurred during part of the dry season (December to February) (fig. 3.3a). The total mortality rate (*K*) did not explain the fluctuation in coffee leafminer density ($r^2 = 0.37$; $P = 0.20$; n = 14). The mortality factor contributing the most to total coffee leafminer mortality was larva death by FOPP (fig. 3.3b), followed by larval and pupal predation (fig. 3.3b, c), but only larval mortality due to predation ($P = 0.01$) and FOPP ($P < 0.01$) significantly contributed to total mortality (fig. 3.4).

Contribution of natural enemies to coffee leafminer mortality at two elevations and rainfall levels. Total mortality was highest at high elevation independently of rainfall level, followed by low elevation under high rainfall, and low elevation under low rainfall ($G = 137.25$, df = 3, $P << 0.001$) (fig. 3.5). Egg predation contributed the most to total mortality rates independently of elevation and rainfall levels (fig. 3.5); independently of rainfall level, egg predation contributed >90% of total mortality at high elevation, and 48-64% at low elevation. Larval parasitism accounted for <1.5% of total mortality at high elevation independently of rainfall level, while it was 12-18% at high elevation (fig. 3.5).

At the low elevation farm, coffee leafminer total mortality was lower during the low rainfall period (0.78) versus the high rainfall period (0.89) ($G = 27.73$, df = 1, P << 0.001) (table 3.3). Egg (0.37) and pupa (0.15) predation were the main sources of real mortality under low rainfall (table 3.3a); while, egg predation (0.57) and death by FOPP (0.11) were the main sources under high rainfall (table 3.3b). The remainder of mortality factors at both rainfall levels contributed ≤0.10 to total real mortality. The main sources of coffee leafminer apparent mortality (i.e. within coffee leafminer stages) at both low and high rainfall were egg predation (0.37 at low, and 0.57 at high rainfall), larval parasitism (0.18, 0.20), and pupal predation (0.39, 0.48) (table 3.3a). Egg (0.13 under low, and 0.15 under high rainfall) and pupal (0.14 under low, and 0.10 under high rainfall) predation contributed the most indispensable mortality independently of rainfall (table 3.3).

At the high elevation farm, coffee leafminer total mortality was similar during low and high rainfall 0.96, 0.97, respectively ($G = 0.69$, df = 1, P = 0.406) (table 3.4). Egg predation was the main source of real mortality independently of rainfall level (0.87 under both high and low rainfall) (table 3.4). The remainder of mortality factors at both elevations and rainfall levels contributed ≤0.10 to total real mortality. Under both low and high rainfall, the main sources of apparent mortality were egg predation (0.87 under low, and 0.86 under high rainfall), larva death due to FOPP (0.32, 0.41), and pupal predation (0.28, 0.41) (table 3.4a). Egg predation was the main source of indispensable mortality independently of the rainfall level (0.30 under low, and 0.22 under high rainfall); the remaining factors each contributed ≤0.02 (table 3.4).

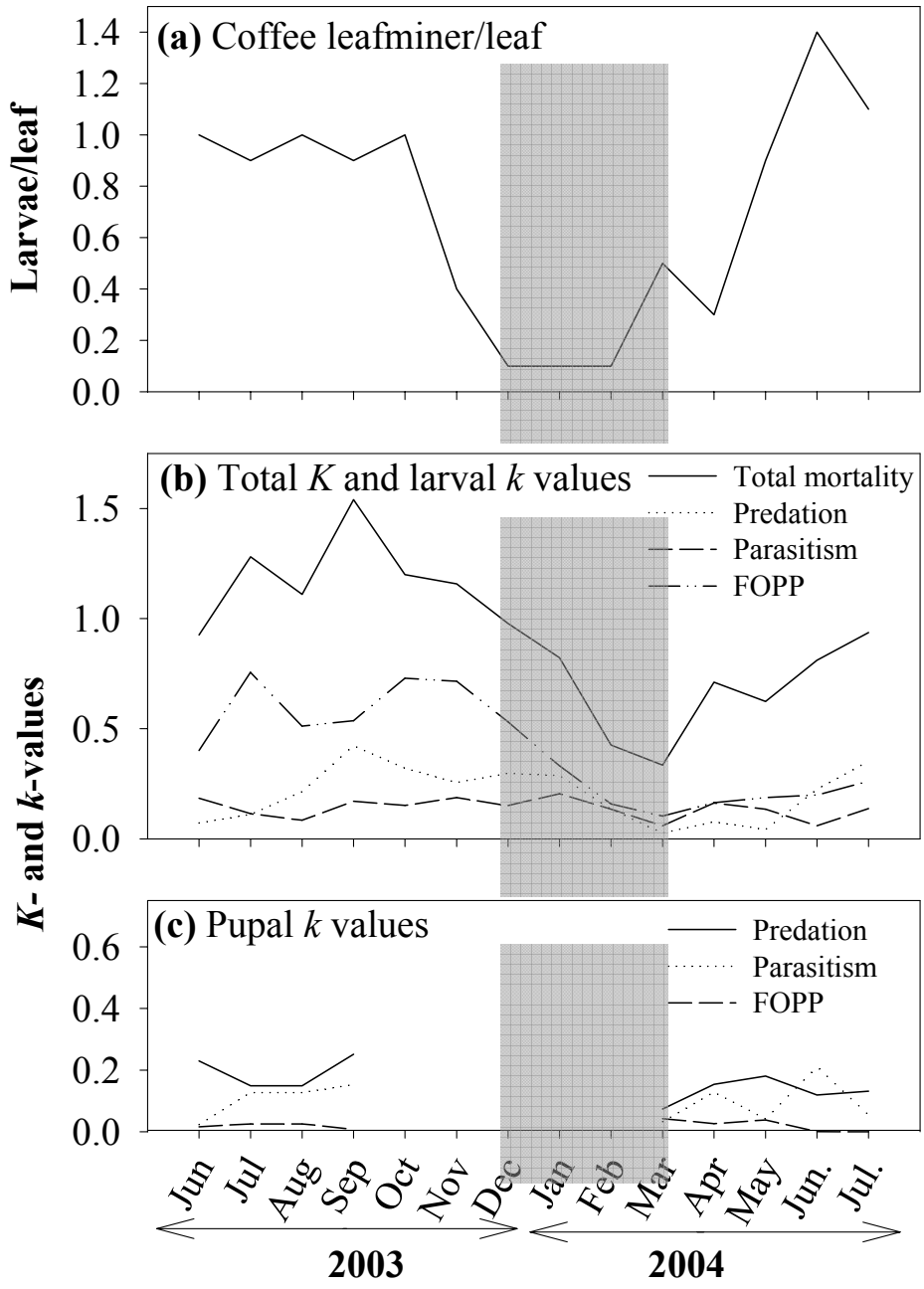

Fig. 3.3. Coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) population dynamics (a) and dynamics of total mortality (b), and mortality due to predation, parasitism, and factors other than predation or parasitism (FOPP) in larvae (b) and pupae (c) at a low elevation farm (El Encanto, 480 m), Cacahoatán, Chiapas, Mexico. The dry season was between December 2003 and March 2004 and is indicated by shading.

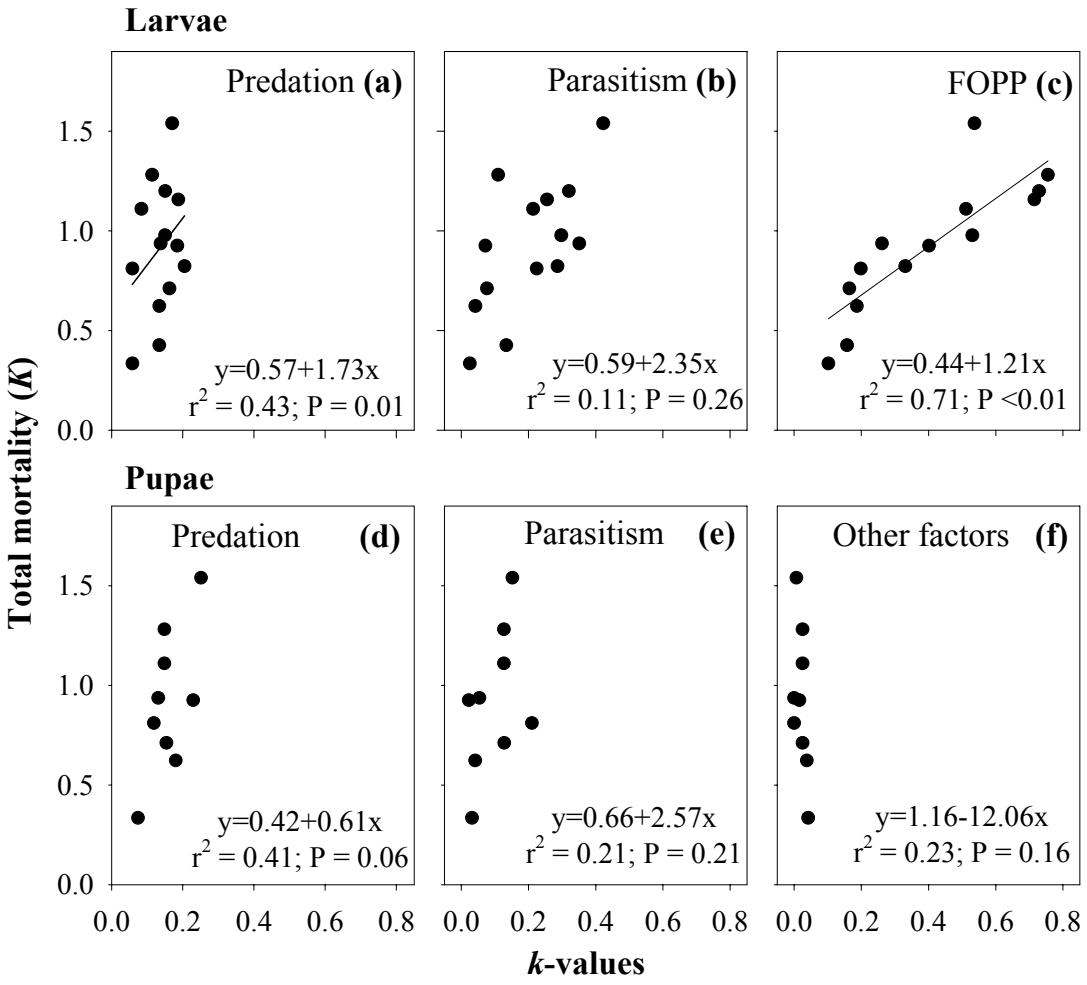

Fig. 3.4. Relationship between individual mortality factors (*k*) and total mortality (*K*) in coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) larvae (a, b, c) and pupae (d, e, f) due to predation (a, d), parasitism (b, e), or factors other than predation or parasitism (FOPP) (c, f) in a low elevation coffee farm (El Encanto, 480 m), Cacahoatán, Chiapas, Mexico, June 2003-July 2004.

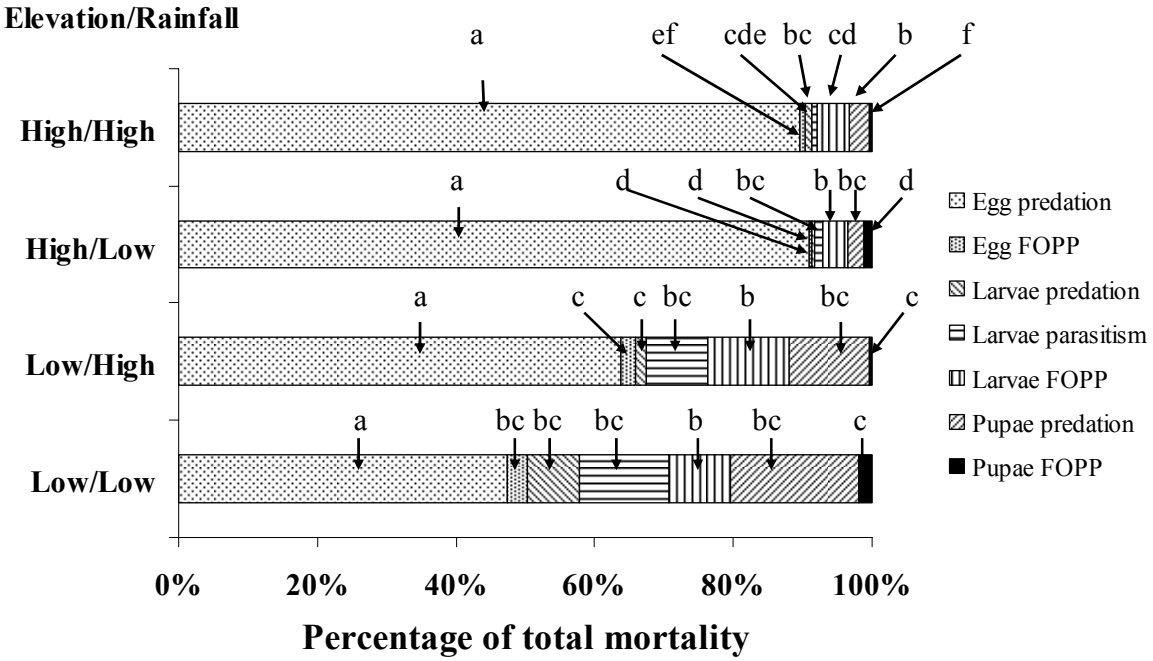

Fig. 3.5. Mortality factors acting on coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) at two elevations and two rainfall levels in Cacahoatán, Chiapas, Mexico. Mortality factors were predation, parasitism, or death due to factors other than predation or parasitism (FOPP) in a low elevation (La Gloria, 550 m) or high elevation (Alpujarras, 960 m) coffee farm, during a period of low rainfall (April, 2005, <100 mm) or high rainfall (June, 2005, >400 mm). *G* tests and Tukey-type multiple comparisons tests were used for comparisons among proportions of mortality due to parasitoids, predators, or FOPP on coffee leafminer eggs, larvae, and pupae within each elevation and rainfall level. Portions of bars within each elevation and rainfall level do not differ significantly if the same letter appears above the segment.

Table 3.3. Real, apparent, and indispensable mortality rates of coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) eggs, larvae, and pupae corresponding to predation, parasitism, or death due to factors other than predation or parasitism (FOPP) in a low elevation coffee farm (La Gloria, 550 m), Cacahoatán, Chiapas, Mexico, during a period of (a) low rainfall (April, 2005, <100 mm), or (b) high rainfall (June, 2005, >400 mm).

Stage	Mortality factor	Initial # of insects	# of deaths	Mortality		
				Real	Apparent	Indispensable
(a)	**Low rainfall**					
Egg	Predation	677	252	0.372	0.37	0.13
	FOPP	425	15	0.022	0.04	0.01
Larva	Predation	410	40	0.059	0.10	0.02
	Parasitism	370	68	0.100	0.18	0.05
	FOPP	302	48	0.071	0.16	0.04
Pupa	Predation	254	98	0.145	0.39	0.14
	FOPP	156	10	0.015	0.06	0.02
Total	—	—	531	0.784	0.78	—
(b)	**High rainfall**					
Egg	Predation	678	385	0.568	0.57	0.15
	FOPP	293	12	0.018	0.04	0.01
Larva	Predation	281	10	0.015	0.04	<0.01
	Parasitism	271	53	0.078	0.20	0.03
	FOPP	218	71	0.105	0.33	0.05
Pupa	Predation	147	70	0.103	0.48	0.10
	FOPP	77	2	0.003	0.03	<0.01
Total	—	—	603	0.89	0.89	—

Table 3.4. Real, apparent, and indispensable mortality rates of coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) eggs, larvae, and pupae corresponding to predation, parasitism, or death due to factors other than predation or parasitism (FOPP) in a high elevation coffee farm (Alpujarras, 960 m), Cacahoatán, Chiapas, Mexico, during a period of (a) low rainfall (April, 2005, <100 mm), or (b) high rainfall (June, 2005, >400 mm).

Stage	Mortality factor	Initial # of insects	# of deaths	Mortality		
				Real	Apparent	Indispensable
(a)	**Low rainfall**					
Egg	Predation	697	606	0.869	0.87	0.30
	FOPP	91	2	0.003	0.02	<0.01
Larva	Predation	89	3	0.004	0.03	<0.01
	Parasitism	86	7	0.010	0.08	<0.01
	FOPP	79	25	0.036	0.32	0.02
Pupa	Predation	54	15	0.022	0.28	0.02
	FOPP	39	8	0.011	0.21	0.01
Total	—	—	666	0.956	0.96	—
(b)	**High rainfall**					
Egg	Predation	488	422	0.865	0.86	0.22
	FOPP	66	4	0.008	0.06	<0.01
Larva	Predation	62	4	0.008	0.06	<0.01
	Parasitism	58	4	0.008	0.07	<0.01
	FOPP	54	22	0.045	0.41	0.02
Pupa	Predation	32	13	0.027	0.41	0.02
	FOPP	19	2	0.004	0.11	<0.01
Total	—	—	471	0.965	0.97	—

Discussion

The coffee leafminer natural enemy complex in the Soconusco region of Chiapas, Mexico, is composed of at least 22 parasitoid and 16 predator species or morphospecies. All parasitoids were parasitic on coffee leafminer larvae, and egg and pupal parasitoids were not recovered. Ants (Formicidae) were the most frequently observed predators of sentinel coffee leafminer eggs, larvae, and pupae, and were the most frequently present predators on coffee plants. Coffee leafminer incidence was higher during the rainy versus the dry season, but total mortality (*K)* did not account for differences in coffee leafminer density between wet and dry months. Analysis of *k*-values, which included only larval and pupal mortality rates, showed that the dynamics of coffee leafminer total mortality were influenced most by death due to FOPP, and larva death due to predation. Total real mortality (from egg through larvae) was higher at high elevation independently of rainfall level relative to low elevation, and higher under high rainfall versus low rainfall at low elevation. Life table analyses showed that independently of elevation and rainfall, egg predation yielded the highest real and indispensable (except at low elevation and low rainfall) mortality. Predation generally was the most important source of egg and pupa apparent mortality, and death by FOPP or parasitism were the most important sources of larval apparent mortality.

The natural enemy diversity observed in this study was higher than previously reported for coffee leafminer (Villacorta, 1980; Flórez & Hernández, 1981; Aranda-Delgado, 1986; Gallardo, 1988; Tozatti & Gravena, 1988; Campos *et al.*, 1989; Mendoza, 1995). *Neochrysocharis*, *Stiropius*, *Closterocerus*, *Pnigalio*, and

Zagrammosoma were the parasitoid genera most frequently collected (table 3.1). Some species, such as *Stiropius letifer* (Mann) and *Pnigalio sarasolai* De Santis have been reported only on coffee leafminer (Whitfield, 1988; Noyes, 2003), but other species, such as *Closterocerus cinctipennis* Ashmead and *Zagrammosoma multilineatum* (Ashmead) have broad host ranges (Noyes, 2003). Most parasitoid species could not be identified with the available keys, and it is likely that undescribed species were present in the study area. The biology of coffee leafminer parasitoids is poorly known; overall, 18 of the species recovered were primary parasitoids, and four were likely facultative secondary parasitoids (table 3.1).

Coffee leafminer predation has been less studied than parasitism, and previous studies did not consider predation of eggs or pupae (Villacorta, 1980; Tozatti & Gravena, 1988; Reis *et al*., 2000). Previous studies conducted in Brazil, Colombia, Ecuador, and Peru recorded 11 species of Vespidae, one genus of Formicidae, and two of Chrysopidae preying on coffee leafminer larvae, but pupal and egg predators were not recorded (Enriquez *et al*., 1975; Parra *et al*., 1977; Tozatti & Gravena, 1988; Mendoza, 1995; Fragoso *et al*., 2002; Carvalho *et al*., 2002; Fernández & Bueno, 2002; Carvalho *et al*., 2005). Ants were the coffee leafminer predators most frequently encountered in the present study (table 3.2). At least four species in four ant genera were observed preying on coffee leafminer eggs, four in two ant genera on larvae, and six in four ant genera on pupae (fig. 3.2). *Camponotus* sp. 1, *Pseudomyrmex* sp. 3 and sp. 2, and *Azteca* sp. were the species most frequently observed preying on coffee leafminer. Quantitative data on the effects of predators on coffee leafminer dynamics, available only from Brazil,

suggested that predatory wasps (Vespidae) are the main source of coffee leafminer mortality (Tozatti & Gravena, 1988; Villacorta, 1980; Reis, *et al*., 2000). However, predatory wasps did not appear to be important mortality factors in the present study. Only once was a predatory wasp observed preying on coffee leafminer larvae in numerous field observations in the Soconusco region between 2003 and 2005 (JRLF, unpubl. data), including 32 h of observation on sentinels, in contrast with 78 predation events by ants during observation on sentinels. Thus, predatory ants in Soconusco region coffee farms may be ecologically equivalent to vespid wasps in Brazilian farms. However, it is unclear whether studies conducted on predation of coffee leafminer in Brazil, or elsewhere, considered or focused on ants. Moreover, the differences in predator species composition also could be due to management practices, because Brazilian coffee production is mainly under sun conditions and farms are intensively managed, while Chiapas production is under shade conditions and farms are traditionally managed. As suggested in prior studies, management practices likely affect both the microclimatic conditions and biodiversity present in coffee farms (Monterrey *et al*., 2001; Nestel *et al*., 2004. Philpott, 2005). Thus, differences between the present study and Brazilian studies could be due to differences in coffee farm microclimatic conditions and/or management regimes, but this needs to be tested.

Ants are the most diverse and abundant organisms in tropical habitats, making up >85% of arthropod biomass in forest canopies (Davidson *et al.*, 2003), and may be the most important source of insect mortality in tropical rainforests (Way & Khoo, 1992; Floren *et al.*, 2002; Armbrecht & Perfecto, 2003). Moreover, ants are one of the most

abundant groups of arthropods in coffee farms (Ramos-Suarez *et al.*, 2002; Armbrecht & Perfecto, 2003; Philpott & Foster, 2005), where they likely are important sources of herbivore mortality (Lachaud *et al*., 1995; Bustillo *et al*., 2002). Studies in Chiapas coffee farms uncovered at least 26 ant species associated with coffee plants, most of them with predatory habits (Armbrecht & Perfecto, 2003; Philpott & Foster, 2005). While those studies did not investigate the impacts of ants on populations of coffee pests, the present study reported at least 13 predatory ant species on coffee plants, of which only one, *Brachymyrmex* sp. was not observed predating on coffee leafminer. The results of the present study thus support suggestions (e.g., Perfecto & Snelling, 1995; Philpott & Foster, 2005) that ants are important predators of coffee pests.

Previous studies suggested that the importance of parasitism as a source of mortality in herbivore insects, including leafminers, is greater in temperate than in the tropical zones, while the importance of predation is greater in the tropics (Rathcke & Price, 1976; Hawkins *et al*., 1997; Queiroz, 2002). Some hypotheses explaining why predation may be more important than parasitism in the tropics have been formulated. For example, Rathcke & Price (1976) formulated the "predation hypothesis," which predicts that herbivores will suffer high predation in the tropics because of a greater diversity of invertebrate and vertebrate predators. Gauld *et al*. (1992) formulated the "nasty host hypothesis," which predicts that herbivores will suffer less parasitism in the tropics because their hosts are more toxic than the extra-tropical hosts. While these hypotheses seek to explain why parasitism or predation pressures vary from the equator to the north, they give elements to support the results of the present study where coffee

leafminer predation was greater relative to parasitism in the study area. While several studies suggested that parasitism was the main mortality factor determining coffee leafminer distribution and abundance (e.g., Flórez & Hernández, 1981; Gravena, 1983; Aranda-Delgado, 1986; Campos *et al*., 1989; Paliz & Mendoza, 1993), the results of the present study suggested that in the Soconusco region predation is more important than parasitism. However, though the results of the present study showed that total mortality due to parasitism represented ~1/4 of that due to predation, it is likely that the real impact of parasitoids was not fully quantified in the present study. Under field conditions, predation of previously parasitized coffee leafminer (e.g., Reis *et al*., 2000), and destructive parasitoid host feeding (Urbaneja *et al*., 2001) are difficult to quantify, and likely affected the parasitoid contribution to total coffee leafminer mortality as measured in this study.

K-factor analysis, which did not include egg mortality in the present study, suggested that larval predation and death by FOPP were the factors that most contributed to total coffee leafminer mortality, while life table analyses, which included all stage-specific sources of mortality, suggested that predation of eggs was the main source of coffee leafminer mortality. Overall, the results of this study are additional evidence that predation, particularly of eggs and pupae, and larval death by FOPP are the main sources of coffee leafminer mortality in the study area.

Weather conditions impacted coffee leafminer distribution and abundance, and natural enemies in previous studies (Reis *et al*., 1976; Villacorta, 1980; Nestel *et al*., 1994). Life table results in the present study showed that total mortality rates were

higher in the high versus the low elevation farm, and that at high elevation mortality rates were not affected by rainfall levels, while at low elevation mortality rates were higher under high versus low rainfall. A study related to the present one (chapter V) showed that rainfall levels and patterns were similar in high and low elevation farms, but average temperatures were significantly higher in the low elevation farm. Together, these results suggested that lower temperatures may largely explain the lower coffee leafminer densities in the high elevation farm (see also chapter V). Lower temperatures in the high elevation farm likely lengthen coffee leafminer developmental times, and thus exposure to natural enemies, increasing the likelihood of predation and parasitism.

Overall, the results of the present study showed that predation of eggs and pupae, and death of larvae due to factors other than parasitoids and predators (FOPP) were the main sources of coffee leafminer mortality in the Soconusco region of Chiapas, Mexico, and suggested that ants were the main source of coffee leafminer egg and pupa mortality. These results are in line with hypotheses pointing to ants as the most important mortality factor affecting herbivore populations in tropical systems (e.g., Way & Khoo, 1992). Moreover, some studies point to enhancing ant populations to increase predation rates and reduce pest population densities in coffee farms (Philpott & Foster, 2005). At the same time, however, other studies showed that ants interfered with the actions of other natural enemies, causing increases of pest populations, and suggested eliminating ants to enhance biological control (Reimer *et al*., 1993; Infante *et al*., 2003). The results of the present study point to the potential of using ants to enhance biological control of coffee leafminer. Clearly, more study is warranted to determine the net impact of ants on pests

and herbivores, generally, including coffee leafminer, in Chiapas coffee farms before advocating their manipulation within the framework of evolving IPM programs for coffee.

CHAPTER IV

COFFEE LEAFMINER *Leucoptera coffeella* (LEPIDOPTERA: LYONETIIDAE) PARASITOIDS IN THE NEOTROPICAL REGION

Introduction

Leafmining insects, whose larvae dig into and feed on leaf tissue, typically have rich communities of natural enemies (Maier, 1988; Hespenheide, 1991; Gencer, 2002; LaSalle & Parella, 2005), in part because their larval feeding habit makes them relatively sessile, and so accessible hosts for parasitoids (Cornell & Hawkins, 1995). Results from previous studies conducted on coffee leafminer *Leucoptera coffeella* (Guérin-Ménèville) (Lepidoptera: Lyonetiidae) in the New World support prior observations pointing to a high diversity of natural enemies associated with leafmining insects. Overall, the list of coffee leafminer natural enemies in the New World includes at least 10 predatory wasps (Vespidae), many larval parasitoids, mostly Eulophidae and Braconidae, and some secondary parasitoids.

Coffee leafminer is an African species [known also as *Perileucoptera coffeella* (Guérin-Ménèville), in the Brazilian literature] that is an important pest in New World coffee producing countries, such as Brazil, Colombia, Cuba, Guatemala, Peru, and Puerto Rico (Mendoza, 1995; Cárdenas & Posada, 2001; Monterrey *et al.,* 2001; Pereira *et al.,* 2003; Fernándes *et al.,* 2004). Yield losses between 50% and 75% due to coffee leafminer were reported in Brazil and Guatemala (Fernández, 1978; Parra *et al*., 1985).

At present, Noyes (2003) provides the most complete compilation of coffee leafminer parasitoids, though limited to species of Chalcidoidea. Noyes (2003) listed 22 coffee leafminer parasitoid genera and 20 species, all in the family Eulophidae. Other studies providing records of non-Chalcidoidea parasitoids of coffee leafminer add at least seven Braconidae species (Le Pelley, 1973; Villacorta, 1980; Aranda-Delgado, 1986; Gallardo, 1988; Flórez & Hernández, 1989; Mendoza, 1995; Penteado-Dias, 1999). Coffee leafminer natural enemy surveys were conducted in Brazil (Parra *et al.,* 1977; Souza, 1979; Mendoza, 1995; Fragoso *et al.,* 2001; Fernández & Bueno, 2002), Colombia (Flórez & Hernández, 1982; Mendoza, 1995), Peru (Enriquez *et al.,* 1975), Ecuador (Mendoza, 1995), Cuba (Konnorova, 1987), and Puerto Rico (Gallardo, 1988; Mendoza, 1995; Sequeira & Hidalgo, 1979). Aranda-Delgado (1986) is the only reference available on coffee leafminer parasitoids for Mexico, and lists 14 genera and four species of parasitoids in Veracruz state, and nine genera and four species in Chiapas state.

The compilation by Noyes (2003) appears to be based primarily on catalogues and international scientific journals, and includes only few regional publications (e.g., technical reports, theses, coffee production manuals, symposium proceedings, and others). Moreover, some of those studies were not formally published, e.g., Aranda-Delgado (1986). Currently, catalogs and/or taxonomic identification keys for coffee leafminer parasitoids are not available, and the lack of such documents slows down studies on coffee leafminer parasitoids, biological control, and management of this pest. In addition, substantial changes in the nomenclature of various parasitoid taxa associated

with coffee leafminer have been made in recent years, thus some records need to be updated. Therefore, the objectives of this study were to compile an annotated checklist of coffee leafminer parasitoids, and construct a key for identifying the genera of parasitic Hymenoptera associated with coffee leafminer in the Neotropical region. These objectives were addressed through a literature review, field collection in Chiapas, Mexico, and examination of previously collected specimens from the Mexican states of Veracruz and Chiapas.

Material and methods

Literature references. Noyes' (2003) database was used as a basis for generating a checklist of the Neotropical coffee leafminer parasitoids. Additional references were identified using literature databases, such as *Agricola*, *Agris*, and *Zoological Records*, and also regional catalogues, theses from Brazil, Puerto, Rico, and Mexico, technical reports, coffee production manuals, conference proceedings, special reports, taxonomic revisions, and other publications from countries or territories within the Neotropical region, as available. These are listed as they are drawn upon in the text.

Specimens from field collections. Field collections consisted mostly of monthly surveys in the summers of 2003, 2004, and 2005, in three coffee farms in the municipality of Cacahoatán, Chiapas, Mexico: El Encanto (14^{o} 59' 21'' N; 92^{o} 09' 55'' W; elevation 480 m); La Gloria (15^{o} 00' 07'' N; 92^{o} 09' 24'' W; 550 m), and Alpujarras (15^{o} 04' 24'' N; 92^{o} 10' 11'' W; 960 m) (fig. 4.1). In addition, collections were made in other coffee farms in the municipalities of Tapachula and Tuxtla Chico, Chiapas, to

recover as many parasitoid species as possible. Leaves with active coffee leafminer mines, i.e. with either coffee leafminer or parasitoid immature stages, were collected, and dissected under a microscope at 25×. Leaves were held in Petri dishes until a parasitoid or coffee leafminer adult emerged. Adult parasitoids were killed in 80% alcohol, and pinned for subsequent identification.

Previously collected specimens from Mexico. Coffee leafminer parasitoid specimens were borrowed from the Insect Collection of El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, and from the private collection of Dr. Eduardo Aranda-Delgado (Terranova Lombricultores, Xalapa, Veracruz.) Specimens making up the latter collection were originally collected by Aranda-Delgado in Veracruz and Chiapas, Mexico, and identified by Dr. C. M. Yoshimoto (Biosystematics Research Institute, Ontario, Canada) (Aranda-Delgado, 1986). The specimens from the ECOSUR collection were from the Soconusco area of Chiapas, Mexico, and had not been identified.

Identification. All parasitoid specimens collected in Chiapas and Veracruz were identified to genus using the keys of Gibson *et al.* (1997), Burks (2002, 2003), and Wharton *et al.* (1997). Species within each genus were identified using specific keys, as noted below.

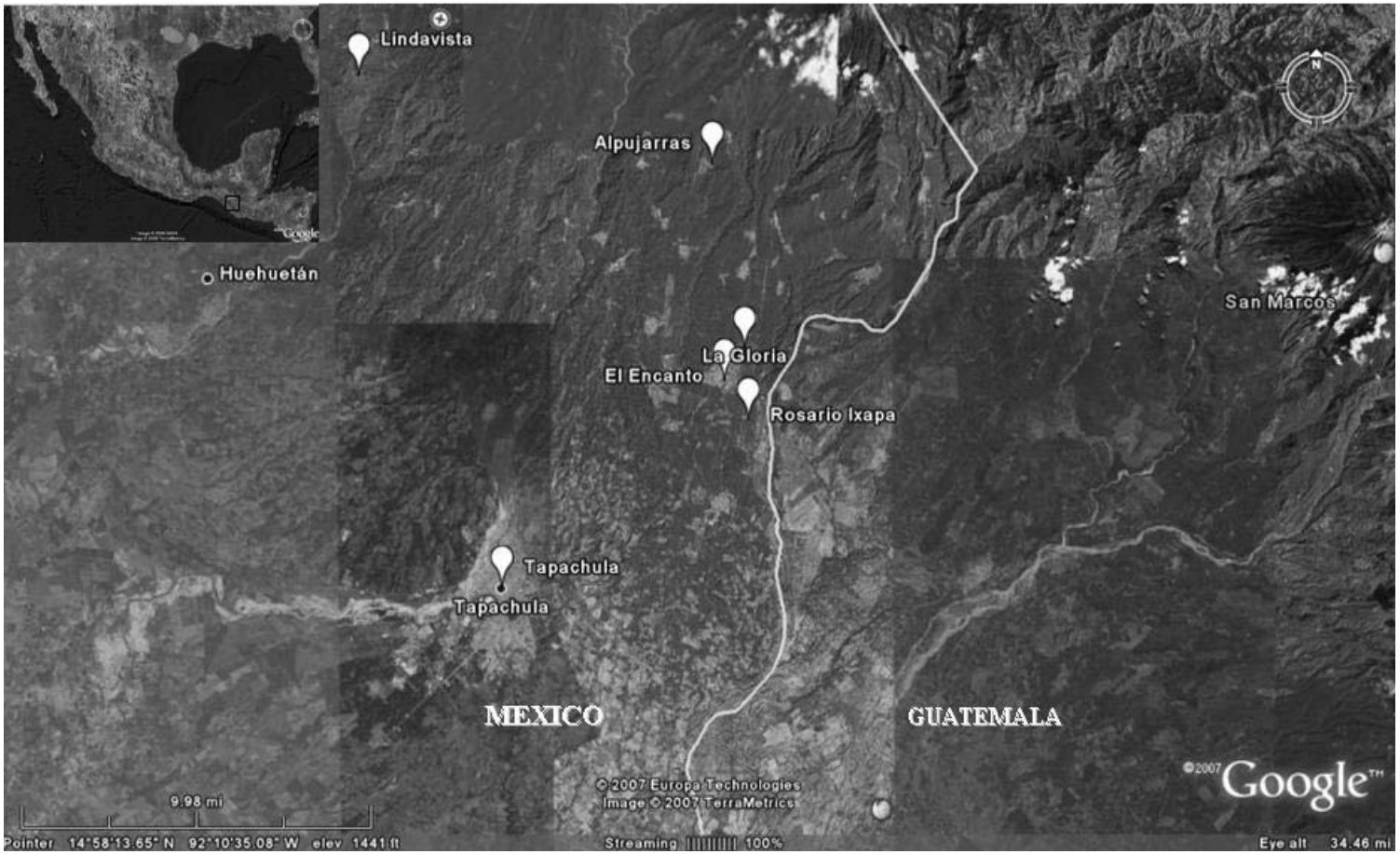

Fig. 4.1. Map of the study area, showing the location of coffee farms where parasitoids collections were made in the municipalities of Cacahoatán (Alpujarras, La Gloria, El Encanto), Tapachula (Tapachula, Lindavista), and Tuxtla Chico (Rosario Ixapa), Chiapas, Mexico (Map from Google Earth http://www.google.com/, March-16, 2007).

Genera and species included in the checklist were listed alphabetically within families. The following information was compiled for each genus, and species as pertinent: valid name, records of association with coffee leafminer, known geographical distribution, and literature references. An identification key to coffee leafminer parasitoid genera was constructed using original descriptions, taxonomic revisions, existing identification keys (Bouček, 1988; Wharton *et al.,* 1997; Schauff *et al.,* 1997, 1998; Burks, 2002, 2003), and observations on specimens collected in Mexico (Aranda-Delgado and ECOSUR collections, this study) and available in the Texas A&M University (TAMU) insect collection. Nomenclature and synonymies were based on Noyes (2003) for Chalcidoidea, and Wharton *et al*. (1997) and Nomina Insecta Nearctica (1998) for Ichneumonoidea, unless indicated otherwise. The morphological terminology follows Gibson (1997) for Chalcidoidea, and Sharkey and Wharton (1997) for Braconidae. Voucher specimens of collections made in Chiapas coffee farms during the present study, and those borrowed from Aranda-Delgado were deposited in the TAMU insect collection (voucher specimens #666). A duplicate set of voucher specimens collected in this study were deposited in the ECOSUR insect collection (without voucher number, but identified as "JRLF-voucher").

Results and discussion

Parasitoids associated with coffee leafminer in the Neotropical region. Coffee leafminer parasitoids were highly diverse in the Neotropics. Overall, 20 genera, and 23 species of Eulophidae, and six genera and seven species of Braconidae (table 4.1) were

associated with coffee leafminer in the Neotropical region. Endoparasitoids were twice more common than ectoparasitoids, at ~66 and ~34%, respectively. The coffee leafminer parasitoid complex was represented almost entirely by larval parasitoids, though one species, *Platocharis coffeae* (Ferrière), was reportedly recovered from leafminer pupae in Africa on other species of coffee leafminers (Kerrich, 1969). Egg parasitoids were not reported (see also chapter III). Most of the Eulophidae genera were primary parasitoids (~72%), followed by facultative parasitoids (13%), and only one species, *Proacrias coffeae* (Ferrière), was reported as a secondary parasitoid (Ihering, 1914). All Braconidae species were primary endoparasitoids on coffee leafminer larvae (table 4.1).

The genera most cited in the literature were the Eulophidae *Cirrospilus* Westwood*, Closterocerus* Westwood*, Horismenus* Walker*, Pnigalio* Schrank*, Proacrias* Ihering, and *Zagrammosoma* Ashmead, and the Braconidae *Mirax* Haliday and *Stiropius* Cameron. Eleven species of Eulophidae and four species of Braconidae were cited only once in the literature (table 4.2). *Zagrammosoma multilineatum* (Ashmead), *Closterocerus lividus* (Ashmead), and *Horismenus cupreus* (Ashmead) were the species of Eulophidae recovered in most countries, and *Mirax insularis* Muesebeck and *Allobracon* spp. were the corresponding species of Braconidae.

Parasitoid species were reported from 17 Neotropical countries or territories (table 4.2). The numbers of parasitoids recorded were highest in Brazil, with 11 genera and 13 species, Mexico, with 13 genera and 11 species, and Puerto Rico, with 14 genera and 10 species, followed by Colombia, Cuba, and Guatemala, with 10, eight, and seven genera, respectively (table 4.2). Brazil was the country with the greatest number of

published studies on coffee leafminer parasitoids, 27 references, and only one (unpublished) study was previously reported from Mexico.

Coffee leafminer parasitoids from Mexico. Ten genera and 23 nominal species of parasitoids were field collected in Chiapas (table 4.3). Few keys were available for the Neotropical species of most of the genera collected, therefore only eight species were identified, and the remaining species were identified as morphospecies. *Closterocerus cinctipennis* Ashmead, *Neochrysocharis arvensis* Graham, *N. chalybea* Hansson, *N. formosa* (Westwood), *Pnigalio sarasolai* (De Santis), and *Stiropius letifer* (Mann) were recorded for the first time associated with coffee leafminer in Mexico. A total of 791 parasitoid specimens were collected during the present study: ~50% were from "El Encanto," the locality with the highest number of parasitoids collected, and the only locality where *Allobracon* sp. was collected. *Neochrysocharis* Kurdjumov was the genus with the highest diversity (six species), and also was the most frequently collected genus (284 specimens). It was followed by *Stiropius* [one species, *S. letifer* (Mann), 113 specimens], *Closterocerus* (three species, 100 specimens), *Pnigalio* (three species, 98 specimens), and *Zagrammosoma* (two species, 64 specimens) (table 4.3).

Table 4.1. Species of parasitoids associated with coffee leafminer *Leucoptera coffeella* (Guérin-Ménèville) in the Neotropical region.

Taxa	Host stage(s) utilized	Ecto/ Endoparasitoid	Primary/Secondary/ Facultative
EULOPHIDAE			
Entedoninae			
Achrysocharoides Girault	Larva	Endoparasitoid	Primary
Chrysocharis nr. *nitetis* (Walker) [1]	Larva	Endoparasitoid	Primary
Chrysonotomyia Ashmead	Larva	Endoparasitoid	Primary
Closterocerus cinctipennis Ashmead	Larva	Endoparasitoid	Primary
C. coffeellae Ihering	Larva	Endoparasitoid	Primary
C. flavicinctus De Santis	Larva	Endoparasitoid	Primary
C. leucopus Ashmead	Larva	Endoparasitoid	Primary
C. lividus (Ashmead)	Larva	Endoparasitoid	Primary
Derostenus Westwood [1]	Larva	Endoparasitoid	unknown
Horismenus aeneicollis Ashmead	Larva	Ectoparasitoid	unknown
H. cupreus (Ashmead)	Larva	Ectoparasitoid	unknown
Neochrysocharis aratus (Walker) [2]	Larva	Endoparasitoid	Primary
N. arvensis Graham [3]	Larva	Endoparasitoid	Primary
N. chalybea Hansson [3]	Larva	Endoparasitoid	Primary
N. formosa (Westwood)	Larva	Endoparasitoid	Facultative
Pediobius Walker [1]	Larva	Endoparasitoid	Facultative
Platocharis coffeae (Ferrière) [1]	Pupa	unknown	Primary
Proacrias coffeae Ihering	Larva	Endoparasitoid	Secondary
Euderinae			
Euderus lividus (Ashmead) [1]	Larva	Ectoparasitoid	Primary
Eulophinae			
Cirrospilus variegatus (Masi) [1]	Larva	Ectoparasitoid	Facultative
Elachertus sp.	Larva	Ectoparasitoid	Primary
Eulophus cemiostomatis Mann	Larva	Ectoparasitoid	Primary
Microlycus Thomson [1]	Larva	unknown	Primary
Miotropis Thomson	Larva	Ectoparasitoid	Primary
Pnigalio nr. *coloni* (Girault) [1]	Larva	Ectoparasitoid	Primary
P. elongatus (Yoshimoto) [1]	Larva	Ectoparasitoid	Primary
P. sarasolai De Santis	Larva	Ectoparasitoid	Primary

Table 4.1. Continued

Taxa	Host stage(s) utilized	Ecto/ Endoparasitoid	Primary/Secondary/ Facultative
Zagrammosoma lineaticeps (Girault)	Larva	Ectoparasitoid	Primary
Z. multilineatum (Ashmead)	Larva	Ectoparasitoid	Primary
Z. seini Wolcott [1]	Larva	Ectoparasitoid	Primary
Tetrastichinae			
Aprostocetus Westwood [3]	Larva	Ectoparasitoid	Facultative
Tetrastichus Haliday	Larva	Ectoparasitoid	unknown
BRACONIDAE			
Braconinae			
Bracon Fabricius	Larva	unknown	unknown
Hormiinae			
Allobracon primus (Muesebeck) [1]	Larva	Endoparasitoid	Primary
Miracinae			
Mirax striata (Penteado-Dias) [1]	Larva	Endoparasitoid	Primary
M. insularis Muesebeck	Larva	Endoparasitoid	Primary
Microgastrinae			
Apanteles Foerster [1]	Larva	Endoparasitoid	Primary
Orgilinae			
Orgilus niger Penteado-Dias	Larva	Endoparasitoid	Primary
O. punctatus (Beyr)	Larva	Endoparasitoid	Primary
Rogadinae			
Stiropius letifer (Mann)	Larva	Endoparasitoid	Primary
S. reticulatus Penteado-Dias [1]	Larva	Endoparasitoid	Primary

[1] Species and/or genus cited only once in the literature.

[2] *Neochrysocharis* were treated as *Closterocerus* in Noyes (2003).

[3] New record as *L. coffeella* parasitoid.

Table 4.2. Parasitoid species associated with coffee leafminer *Leucoptera coffeella* (Guérin-Ménèville) in the Neotropical region by country or territory. Numbers in parenthesis following country/territory names represent the number of genera and species, respectively, recorded in each country or territory. Records marked with * represent new records for Mexico.

	Country or territory (genera/species)																	
TAXA	**Brazil (11, 13)**	**Colombia (10,**	**Cuba (8, 5)**	**Dominica (1, 1)**	**Ecuador (6, 2)**	**El Salvador (1,**	**Grenada (2, 2)**	**Guadalupe (1,**	**Guatemala (7,**	**Honduras (2, 1)**	**Jamaica (1, 1)**	**Mexico (13, 11)**	**Nicaragua (1, 1)**	**Peru (4, 1)**	**Puerto Rico (14,**	**San Vicente (2,**	**Venezuela (3, 2)**	**TOTAL (26, 30)**
EULOPHIDAE																		
Achrysocharoides		√													√			2
Aprostocetus												*						1
Chrysocharis			√									√						2
C. nr. *milleri*												√						1
Chrysonotomyia									√			√			√			3
Cirrospilus	√	√	√		√							√	√					
C. variegates													√					1
Closterocerus	√	√										√						3
C. cinctipennis			√									*			√			2
C. coffeellae	√	√	√															3
C. flavicinctus	√																	1
C. leucopus															√	√		2
C. lividus		√					√								√	√	√	5
Derostenus															√			1
Elachertus	√											√			√			3
Euderus lividus															√			1
Eulophus	√	√	√															3
E. cemiostomatis	√																	1
Horismenus	√		√									√			√			4
H. aeneicollis	√																	1
H. cupreus	√	√	√		√		√		√						√	√		8
Microlycus														√				1
Miotropis			√									√						2
Neochrysocharis												*						1
N. arata														√				1
N. arvensis												*						1
N. chalybea												*						1
N. formosa												*			√			2
Pediobius														√				1

Table 4.2. Continued

TAXA	Brazil (11, 13)	Colombia (10,	Cuba (8, 5)	Dominica (1, 1)	Ecuador (6, 2)	El Salvador (1,	Grenada (2, 2)	Guadalupe (1,	Guatemala (7,	Honduras (2, 1)	Jamaica (1, 1)	Mexico (13, 11)	Nicaragua (1, 1)	Peru (4, 1)	Puerto Rico (14,	San Vicente (2,	Venezuela (3, 2)	TOTAL (26, 30)
Platocharis coffeae															√			1
Proacrias	√		√														√	3
P. coffeae	√	√	√												√			4
Pnigalio		√			√	√			√			√						4
P. nr. coloni												√						1
P. elongatus												√						1
P. sarasolai		√							√			*						3
Tetrastichus	√	√			√										√			5
Zagrammosoma	√	√	√		√				√			√		√	√			8
Z. lineaticeps												√						1
Z. multilineatum	√	√	√								√	√					√	6
Z. seini															√			1
BRACONIDAE																		
Allobracon		√							√	√		*						4
A. primus		√																1
Apanteles									√									1
Bracon									√						√			2
Mirax	√																	1
M. insularis	√			√				√							√			4
M. striata	√																	1
Orgilus	√																	1
O. niger	√																	1
O. punctatus	√																	1
Stiropius	√									√		*						3
S. letifer	√				√					√		*						3
S. reticulatus	√																	1

Table 4.3. Coffee leafminer *Leucoptera coffeella* (Guérin-Ménèville) parasitoids from Mexico. Data shown are species and numbers of specimens recovered from two coffee farms, Alpujarras and El Encanto, in Cacahoatán, and other localities in the Soconusco region of Chiapas, Mexico. Data also include specimens borrowed from a private collection (see text).

Genus/species	Specimens from private collection	Field collections in Chiapas, Mexico Specimens (%) by locality			Total
		Alpujarras N 15° 04' 24'' W 92° 10' 11'' 960m	**El Encanto** N 14° 59' 21'' W 92° 09' 55'' 480m	**Other**[1]	
Eulophidae					
Aprostocetus sp.	2				
Chrysocharis nr *nitetis*	4				
Chrysocharis spp. [2]	6				
Chrysonotomyia sp.	12				
Cirrospilus spp. [2]	5	2 (2.5)	4 (1.0)	15 (4.7)	21 (2.7)
Closterocerus spp.[2]	5	26 (32.9)	11 (2.8)	20 (6.3)	57 (7.2)
C. cinctipennis	12	1 (1.3)	35 (8.9)	7 (2.2)	43 (5.4)
Elachertus spp. [2]	9	2 (2.5)	16 (4.1)	29 (9.1)	47 (5.9)
Horismenus spp.[2]	2	3 (3.8)	8 (2.0)	2 (0.6)	13 (1.6)
Miotropis sp.	0	3 (3.8)	3 (0.8)	0 (0)	6 (0.8)
Neochrysocharis spp. [2]	2	10 (12.7)	98 (24.9)	87 (27.3)	195 (24.7)
N. arvensis	0	1 (1.3)	13 (3.3)	14 (4.4)	28 (3.5)
N. chalybea	0	3 (3.8)	7 (1.8)	13 (4.1)	23 (2.9)
N. formosa	0	1 (1.3)	27 (6.9)	10 (3.1)	38 (4.8)
Pnigalio spp.[2]	2	6 (7.6)	28 (7.1)	39 (12.2)	73 (9.2)
P. sarasolai	0	3 (3.8)	17 (4.3)	5 (1.6)	25 (3.2)
Zagrammosoma sp.					
Z. lineaticeps	1	0 (0)	0 (0)	1 (0.3)	1 (0.1)
Z. multilineatum	4	1 (1.3)	30 (7.6)	32 (10.0)	63 (8.0)
Braconidae					
Allobracon sp.	0	0 (0)	45 (11.5)	0 (0)	45 (5.7)
Stiropius letifer	0	17 (21.5)	51 (13.0)	45 (14.1)	113 (14.3)
TOTAL (specimens)	75	79 (~10%)	393 (~50%)	319 (40%)	791

[1] Includes various coffee farms in the Soconusco region, and specimens deposited in the Insect collection at ECOSUR-Tapachula, Chiapas, labeled only as parasitoids of *L. coffeella* from the Soconusco region, Chiapas, Mexico.

[2] Includes at least two unidentified species.

Key to the genera of coffee leafminer parasitoids in the Neotropical region

The key below was constructed assuming that users have basic knowledge of Hymenoptera anatomical terminology, though it is accompanied with figures showing many of the referred structures. Untrained users will find useful information in Gibson (1997) and Sharkey and Wharton (1997). Although the key includes all parasitoid genera recorded for coffee leafminer in the Neotropical region, special attention must be paid to those followed with a symbol. These represent taxa that were reported in older literature without further confirmation (§), or may currently be placed in a different genus (*), or specimens were not available for confirmation (ψ) (see notes below in the "*Checklist*... section).

1. Forewings with venation complete, with at least 3 closed cells (figs. 4.2a-f). Antenna filiform (Braconidae) ……..……..……..…… 2

\- Wing venation reduced (fig. 4.2g-l). Antenna geniculate (Eulophidae) …….. 7

2(1). Labrum exposed, not concealed by clypeus (fig. 4.3h) ..….....….…..…..... 3

\- Labrum concealed by clypeus (fig. 4.3i) .....….....…….…..... 5

3(2). Epicnemial carina completely absent, hind wing M+CU less than half as long as 1M (fig. 4.2a) ……..…….……..... *Bracon* F. *

\- Epicnemial carina present, at least ventrally, hind wing M+CU almost as long as 1M (fig. 4.2b) ..……..…… …….……....….....…..... 4

4(3). Occipital carina present. Petiole and metasomal terga distinctly sclerotized; tergum 2 with median longitudinal carina (fig. 4.4h) …..… *Stiropius* Cameron

- Occipital carina absent. Petiole partially desclerotized. Metasomal terga desclerotized dorsally ..... *Allobracon* Gahan

5(2) Fore wing with RS vein reaching wing margin as a tubular vein (fig. 4.2d). Occipital carina present, at least laterally ……..… *Orgilus* Ness

- Fore wing with RS vein not reaching wing margin as a tubular vein (figs. 4.2e, f). Occipital carina absent ...…….....……..…… 6

6(5). Antennae with 12 flagellomeres. Metasomal terga at least partially desclerotized dorsally (fig. 4.4i). R1 vein absent (fig. 4.2e) .....…..... *Mirax* Haliday

- Antennae with 16 flagellomeres. Metasomal terga sclerotized dorsally. R1 vein present (fig. 4.2f) .....….....……..... *Apanteles* Haliday §

7(1). Scutellum with one pair of setae (figs. 4.5b, c). Submarginal vein of forewings with 2 dorsal setae ……..… 8

- Scutellum with two or more pairs of setae (figs. 4.5d, h). Submarginal vein of forewings either with one, or more than 2 dorsal setae 17

8(7). Ocellar triangle clearly encircled by sutures (fig. 4.3d). Petiole at least 2× longer than broad …….…..... *Derostenus* Westwood §

- Ocellar triangle not surrounded by sutures. Petiole variable .....…….....….... 9

9(8). Propodeum with shiny, median strip, bordered laterally by depressed and usually sculptured area (fig. 4.4d); petiole reticulate ..... *Horismenus* Walker

- Propodeum with or without median carina, but never with a raised shiny median strip. Petiole not reticulate …...……....……..... 10

10(9). Propodeum with distinct plicae (figs. 4.4e, f) .....…….....…..... 11

- Propodeum without distinct plicae (figs. 4.4a-c) 13

11(10). Frontofacial suture transverse (fig.4.3a). Postmarginal vein longer than the stigmal vein ……. *Platocharis* Kerrich § ψ

- Frontofacial suture V-shaped (figs. 4.3b, e). Postmarginal vein variable 12

12(11). First gastral tergite well-sclerotized, dorsal surface not collapsing in air-dried specimens; first gastral sternite with a strongly sclerotized, reticulate region at the petiolar base ……..……..…… *Pediobius* Walker §

- First gastral tergite collapsing in air-dried specimens; first gastral sternite without sclerotized reticulation at the petiolar base ..... *Proacrias* Ihering

13(10). Postmarginal vein longer than stigmal vein, usually 1.5-2.0× (fig. 4.2i). Petiole distinct ……...…….…….. *Chrysocharis* Förster

- Postmarginal vein shorter than stigmal vein (figs. 4.2h, l). Petiole usually hidden ……...……. 14

14(13). Forewing with two lines of setae extending from stigmal vein (fig.4.2j) ……...…… *Chrysonotomyia* Ashmead

- Forewing with at most one line of setae extending from stigmal vein ……..... 15

15(14). Mesosoma dorsally with pits (fig. 4.5a). Frontofacial sutures nearly straight (fig. 4.3a) ……..……..…….... *Achrysocharoides* Girault

- Mesosoma dorsally without pits (figs. 4.5b-h). Frontofacial suture V-shaped (fig. 4.3b, e) ….…….. 16

16(15). Entire antenna strongly compressed, and scape subtriangularly expanded (fig. 4.4k). Forewings usually with crossed dark bands (fig. 4.2h) ……...

…….. *Closterocerus* Westwood

- Antenna not strongly compressed, and scape no expanded (figs. 4.4l-p). Forewings never with distinct dark bands …..... *Neochrysocharis* Kurdjumov

17(7). Forewing hyaline, with 2-3 rows of setae radiating from the stigmal vein and a bare area in its base, and a group of isolated setae just below the marginal vein (fig. 4.2k). ……. *Euderus* Haliday

- Never with the above combination of characteristics .....…..... 18

18(17). Postmarginal vein less than 1/3 the length of the stigmal vein (fig. 4.2l) 19

- Postmarginal vein more than half the length of the stigmal vein …….…..... 20

19(18). Submarginal vein with one dorsal seta (fig. 4.2l) …….. *Tetrastichus* Haliday

- Submarginal vein with more than two dorsal setae ..... *Aprostocetus* Westwood

20(18). Funicle with two segments (figs. 4.4m-n) ….....……..... 21

- Funicle with three or four segments (figs. 4.4j, l, o-p) 22

21(20). Vertex vaulted between the eyes (fig. 4.3g). Notaulus not extending to posterior margin of mesoscutum (fig. 4.5h) .....……..... *Zagrammosoma* Ashmead

- Vertex not vaulted between the eyes (fig. 4.3c). Notaulus distinct and complete to hind posterior margin of mesoscutum (fig. 4.5d) …..... *Cirrospilus* Westwood

22(20). Funicle with three segments (fig. 4.4l) ……...……..... 23

- Funicle with four segments (figs. 4.4j, o, p) .....……..... 24

23(22). Mandibles reduced, not capable of meeting medially (fig. 4.3f). Middle legs with basitarsus shorter than 2nd tarsal segment ...…..... *Eulophus* Geoffroy

- Mandibles not reduced. Middle legs with basitarsus equal or longer than 2nd tarsal segment ..... *Mycrolicus* Thomson §

24(22). Notauli incomplete. Male funicle with long branches (fig. 4.4p). Propodeum with median carina and distinct lateral plicae (fig. 4.4f) …….. *Pnigalio* Schrank

- Notauli complete (fig. 4.5e). Male funicle without branches. Propodeum with median carina and without lateral plicae (fig. 4.4c) …...……..... 25

25(24). Scutellum with submedian grooves incomplete or absent . *Miotropis* Thompson

- Scutellum with complete submedian grooves (fig. 4.5e) ..… *Elachertus* Spinola

Check list of Eulophidae coffee leafminer parasitoids

Information provided for each genus includes diagnosis, taxonomy, biology, and host records. Diagnoses are based on previous taxonomic studies, which are referred to in parenthesis for each genus. Information relative of number of species [according to Noyes (2003) for Eulophidae], available keys for species, synonymies, and misspellings in relation to coffee leafminer are included in the *Taxonomy* section. Finally, the section *Biology and Host Records* includes information on biology, most common hosts, select literature, and geographic distribution for each parasitoid species previously reared from coffee leafminer.

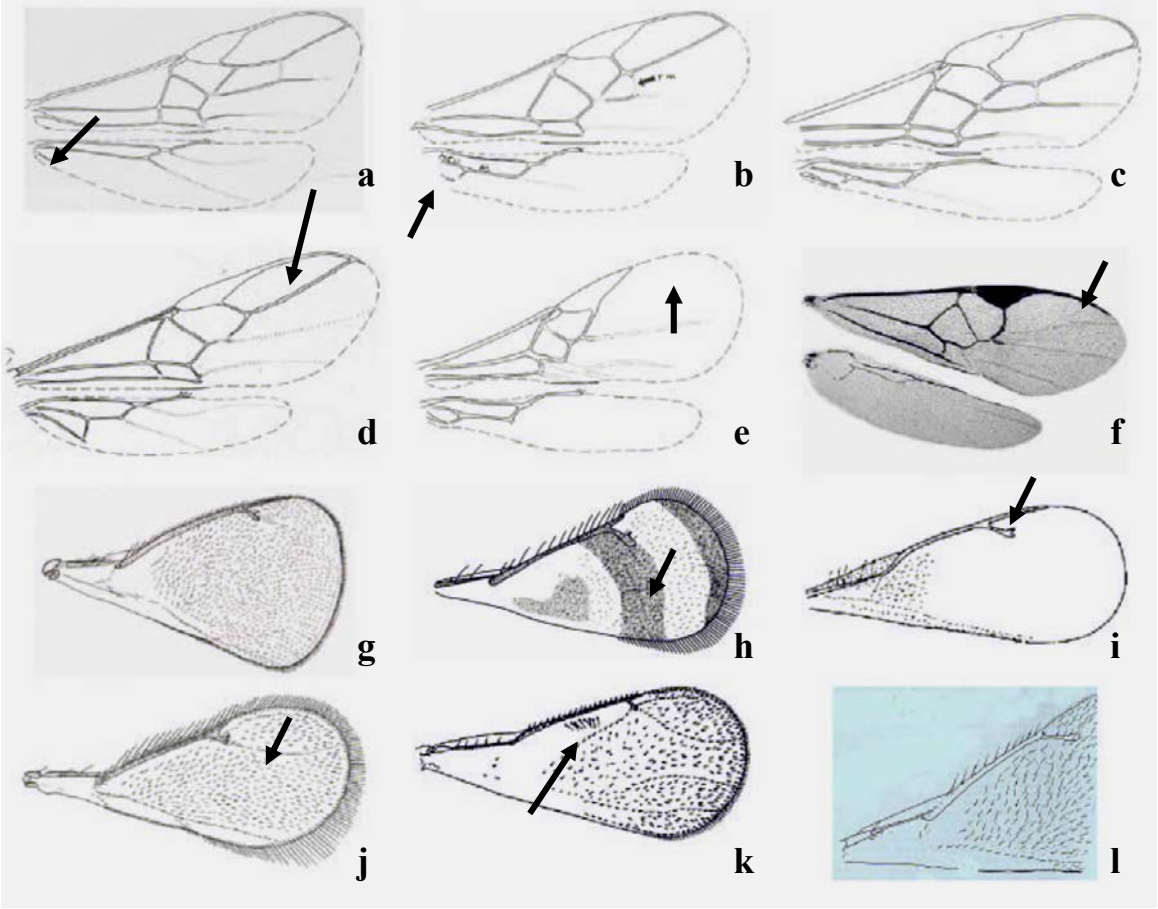

Fig. 4.2. Parasitoid wings. a, *Bracon* (Quicke, 1997); b, *Allobracon* (Whitfield & Wharton, 1997); c, *Stiropius* (Shaw, 1997); d, *Orgilus* (van Achterberg, 1997); e, *Mirax* (Whitfield, 1997b); f, *Apanteles* (González-Hernández *et al*., 2003); g, *Chrysocharis* (Schauff, 1991); h, *Closterocerus* (Schauff, 1991); i, *Elachertus* (Zhu & Huang, 2001); j, *Neochrysocharis* (Schauff, 1991); k, *Euderus* (Coote, 1994); l, *Tetrastichus* (Graham, 1991).

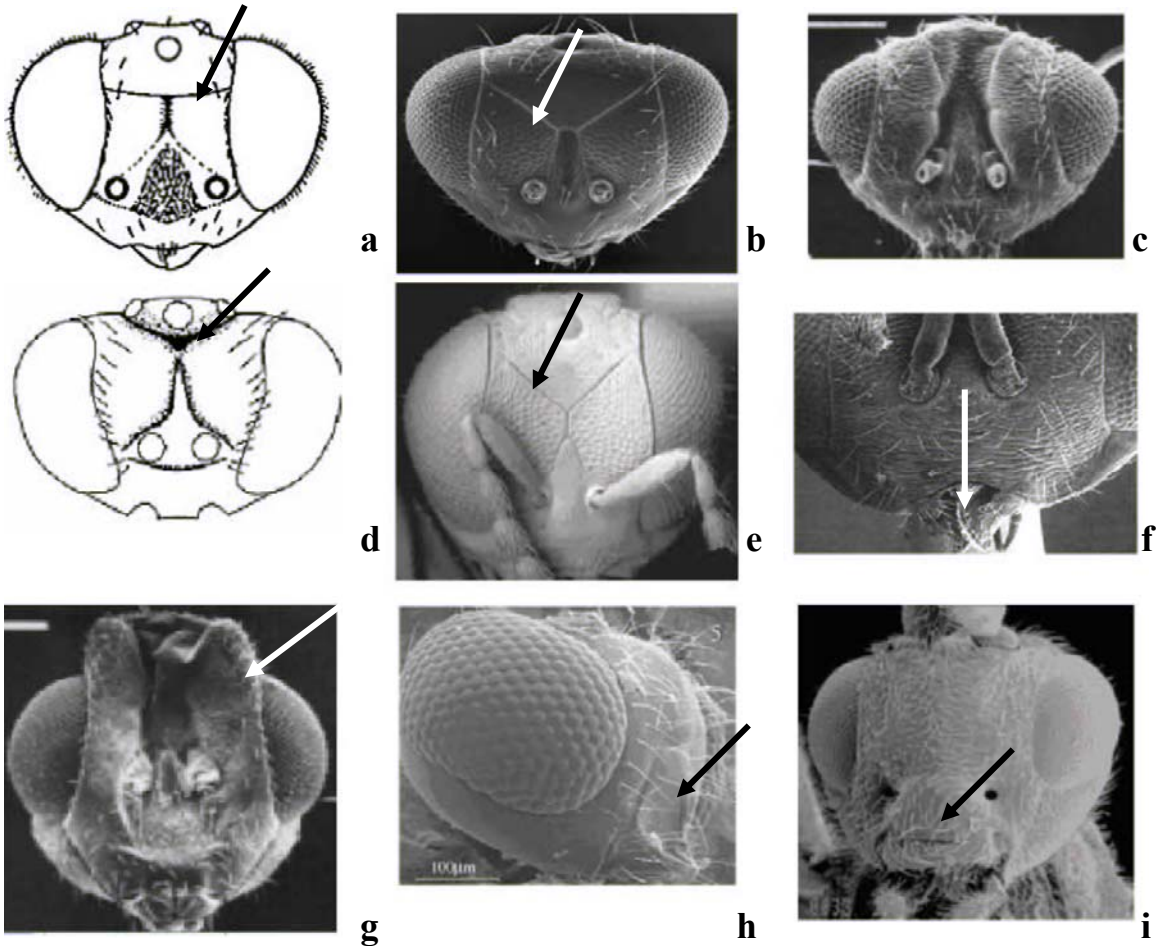

Fig. 4.3. Parasitoid heads. a, *Achrysocharoides* (Schauff *et al.*, 1997); b, *Chrysocharis* (La Salle & Parella, 2005); c, *Cirrospilus:* (La Salle & Parella, 2005); d, *Derostenus* (Hansson, 1986); e, *Horismenus* (Hansson *et al.*, 2004); f, *Eulophus* (Schauff *et al*., 1997); g, *Zagrammosoma* (La Salle & Parella, 2005); h, *Allobracon* (Mitio-Shimbori, 2005); i, *Orgilus* (van Achterberg, 1997).

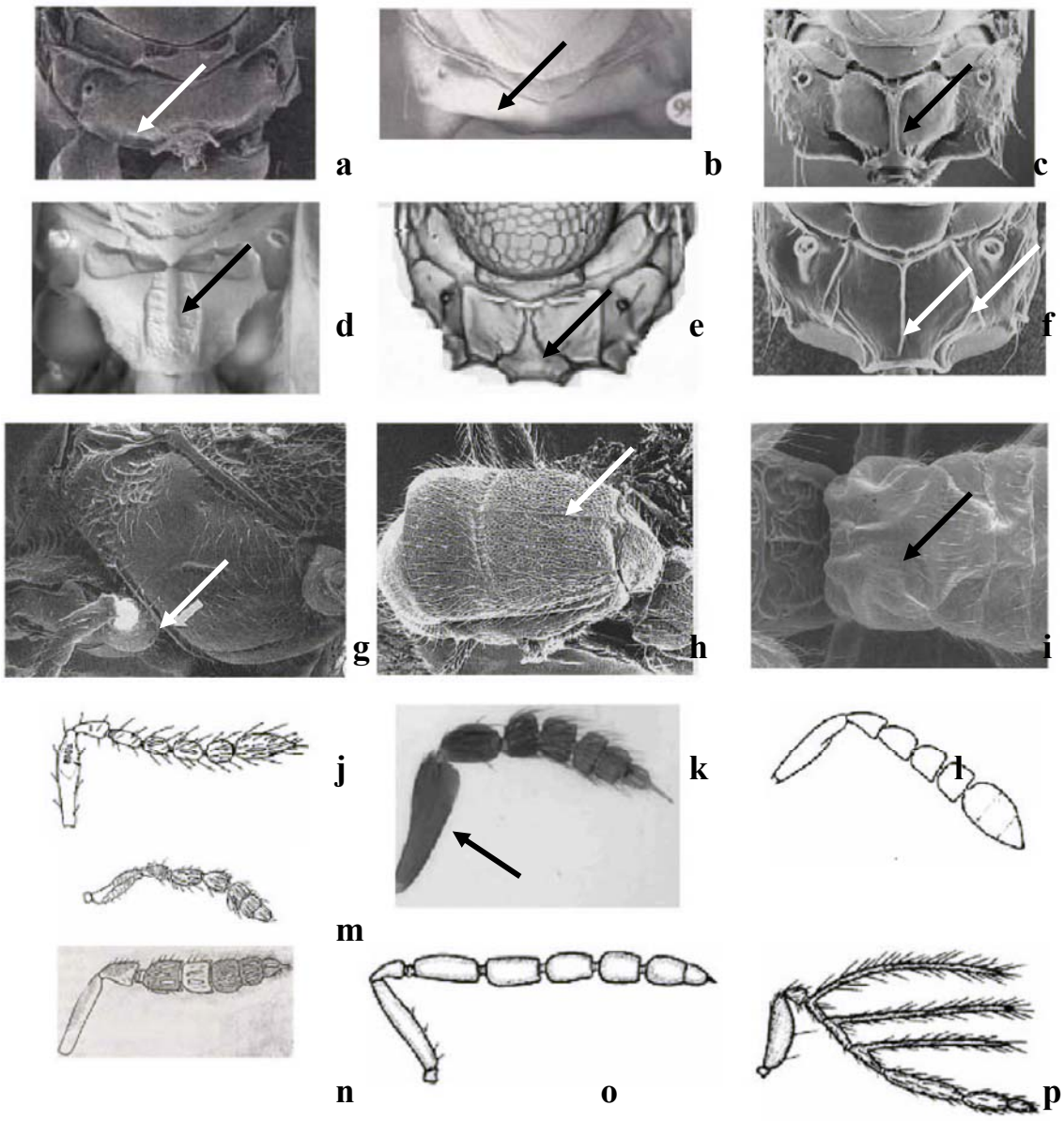

Fig. 4.4. Parasitoid propodea (a-f), mesosternum (g), abdomens (h-i), and antennae (j-p). a, *Achrysocharoides* (Schauff *et al.*, 1997); b, *Chrysonotomyia* (Hannson, 2004); c, *Elachertus* (Schauff, 1985); d, *Horismenus* (Hansson *et al.*, 2004); e, *Pediobius* (Dawah *et al.*, 2002); f, *Pnigalio* (Zhu & Huang, 2003); g, *Clinocentrus* (Whitfield & Wharton, 1997); h, *Stiropius* (Shaw, 1997); i, Mirax (Whitfield, 1997b); j, *Elachertus* (Zhu & Huang, 2001); k, *Closterocerus* (Edwards & LaSalle, 2004); l, *Microlycus* (Kerrich, 1968); m, *Cirrospilus* (Evans, 1999); n, *Zagrammosoma* (LaSalle, 1989); o, *Pnigalio* female, and p, male (Burks, 2003).

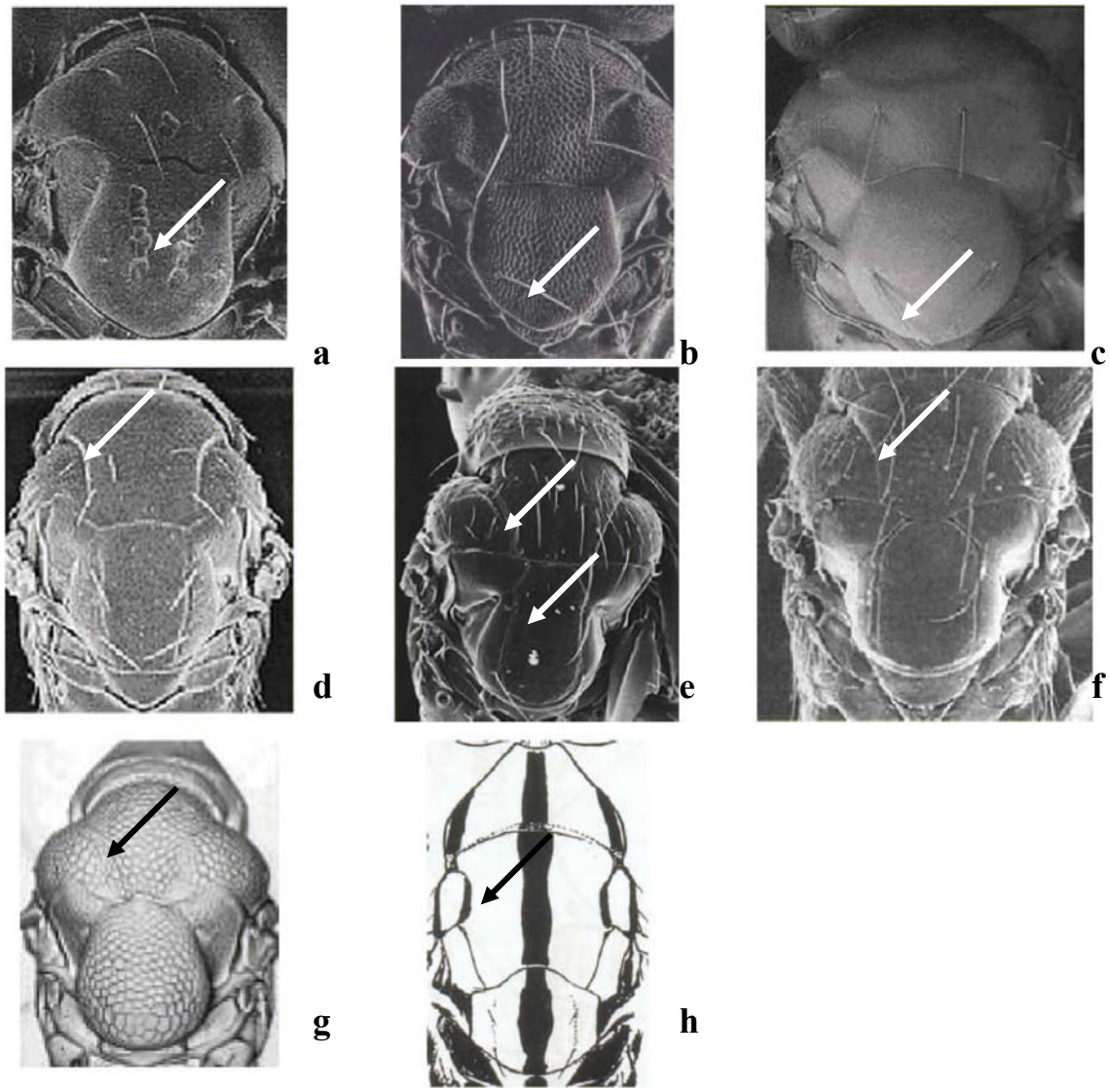

Fig. 4.5. Parasitoid thoraces. a, *Achrysocharoides* (Schauff *et al.*, 1997); b, *Chrysocharis* (Hansson, 1995); c, *Chrysonotomyia* (Hannson, 2004); d, *Cirrospilus* (Zhu *et al.*, 2002); e, *Elachertus* (Schauff, 1985); f, *Miotropis* (Schauff *et al*., 1997); g, *Pediobius* (Zhu & Huang, 2003); h, *Zagrammosoma* (Bouček, 1988).

Achrysocharoides Girault, 1913

Diagnosis (Hansson, 1985b; Schauff *et al*., 1998; Burks, 2003). Head with eyes densely pubescent, frontofacial suture T-shaped, clypeus not delimited by sutures. Flagellum with eight segments. Midlobe of mesoscutum with two pairs of setae. Scutellum with one pair of setae. Mesoscutum and scutellum with pits in some species. Propodeum without median carina or plicae. Petiole wider than long. Forewings with two submarginal setae, and without line of setae radiating from stigmal vein; postmarginal vein shorter than stigmal vein.

Taxonomy. Noyes (2003) included 52 species in this genus. Keys for Neotropical species are not available. Yoshimoto (1977) and Kamijo (1991) presented keys for North American species.

Biology and Host Records. Species are primary endoparasitoids of larvae. Hosts include species from Hymenoptera, Diptera, and Lepidoptera. Kamijo (1991) reported several species reared from leafminers in North America. *Achrysocharoides* sp. was recorded on coffee leafminer in Puerto Rico (Gallardo, 1988) and Colombia (Flórez & Hernández, 1982; Mendoza, 1995).

Aprostocetus **Westwood, 1833**

Diagnosis (Graham, 1987). Head without frontofacial suture. Ocelli surrounded by an impressed line. Antenna with funicle and clava with three segments each. Mesoscutum with median line, and scutellum with submedian grooves and two pairs of setae. Two or more setae present in the submarginal vein of forewings. Postmarginal vein absent. Cercus in metasoma with a single, long seta.

Taxonomy. This genus includes 697 species (Noyes, 2003). Keys for Neotropical species are not available. A key to European species is available in Graham (1987). The species now included in this genus were formerly placed in several other genera, including *Tetrastichus* (Graham, 1987).

Biology and Host Records. Species are primary ectoparasitoids of larvae inhabiting concealed habitats, such as galls, though some are secondary parasitoids (Noyes, 2003). Some species were recorded from Lepidoptera (Noyes, 2003), including Lyonetiidae leafminers other than coffee leafminer (Gates *et al*., 2002). Previous records of this genus associated with *L. coffeella* were not available in the literature, though *A. leucopterae* (Ferrière) was reared in Africa as a primary ectoparasitoid in other *Leucoptera* species, and as a secondary parasitoid on *Eulophus* sp. (Noyes, 2003). Specimens identified in Aranda-Delgado (1986) as *Tetrastichus* run to *Aprostocetus* in the Schauff *et al*. (1997) key because many *Tetrastichus* species were placed in *Aprostocetus* after 1987 (Graham, 1987). *Aprostocetus* is reported herein as a new record for coffee leafminer.

***Chrysocharis* Förster, 1856**

Diagnosis (Hansson, 1985b; Schauff, 1991; Hansson, 1995; Burks, 2003). Head with frontofacial suture V or Y-shaped. The antennal scrobes usually joined before they reach the frontofacial suture. Scrobal grooves do not extend ventrally below toruli. Antenna with three or four funicular, and one or two claval segments. Clypeus not delimited by sutures. Mesoscutum with two or more pairs of setae. Scutellum with one

pair of setae. Forewings strongly wedge-shaped; postmarginal vein elongate, at least twice the length of stigmal vein. Petiole usually longer than broad.

Taxonomy. This is a large genus with at least 135 species (Noyes, 2003). Hansson (1987) provided a key to New World species, and Hansson (1997) a key to Mexican species.

Biology and Host Records. In general, species in this genus are primary endoparasitoids in larval or pupal stage leafminers (Hansson, 1985a; Bouček, 1988). Notley (1948) reported *C. lepelleyi* (Ferrière) as a primary parasitoid on *Leucoptera* species in Africa. *Chrysocharis* nr. *nitetis* (Walker) was reported on coffee leafminer in Mexico (Aranda-Delgado, 1986, as *Chrysocharis* nr. *milleri*), and *Chrysocharis* sp. was reported from Mexico (Aranda-Delgado, 1986) and Cuba (Konnorova, 1987).

***Chrysonotomyia* Ashmead, 1904**

Diagnosis (Burks, 2003; Hansson, 2004). Head with frontofacial suture from straight to V-shaped, antennal scrobes joining frontal suture separately. Subtorular sulci present. Antenna with three or four funicular and two or three claval segments; the apical claval segments more or less fused. Clypeus delimited only laterally. Midlobe of mesoscutum with one pair of setae. Notauli poorly delimited, or missing in posterior part. Scutellum with one pair of setae. Propodeum small, smooth, and shiny; sometimes, with complete median carinae. Petiole usually short. Submarginal vein with two setae. Postmarginal vein usually shorter than the stigmal vein.

Taxonomy. Noyes (2003) listed 126 species in this genus. Hansson (2004) provided a key to 97 Neotropical species, and Yoshimoto (1980) a key to North

American species. Hansson (1990) gave elements to separate *Chrysonotomyia* from *Neochrysocharis*, and many species described under *Chrysonotomyia* were transferred to *Neochrysocharis* and *Closterocerus*. Therefore, it is possible that many records of coffee leafminer parasitoids cited under *Chrysonotomyia* sp. belong to other genera. For example, specimens cited as *Chrysonotomyia* sp. 4 in Aranda-Delgado (1986) were identified in the present study as *Neochrysocharis* sp., and *Chrysonotomyia* sp. 6, 10, and 14 as *Closterocerus* spp. (table 4.3).

Biology and Host Records. This genus includes primary endoparasitoids on gall makers, especially Cecidomyiidae (Diptera). *Chrysonotomyia* sp. was recorded on coffee leafminer in Puerto Rico (Gallardo, 1988), Guatemala (Campos *et al*., 1989; Mendoza, 1995), and Mexico (Aranda-Delgado, 1986); and, *C. coffeae* was recorded from Brazil (Mendoza, 1995), but the latter species was not cited by Noyes (2003). Two parasitoid species with the specific epithet *coffeae* are associated in the literature with coffee leafminer: *Platocharis coffeae* Ferrière from Africa (Kerrich, 1969), and *Proacrias coffeae* Ihering from the Neotropical region (De Santis, 1979); thus it is likely that *Chrysonotomyia coffeae* is a synonym of *Proacrias coffeae*, which in some cases was cited as *Neochrysocharis coffeae* (Bouček, 1977).

***Cirrospilus* Westwood, 1832**

Diagnosis (Schauff *et al*., 1998; Zhu *et al*., 2002; Burks, 2003). Head with frontofacial suture V-shaped, antennal scrobes deep and joining frontal suture separately. Clypeus not set off by sutures. Funicle two-segmented, clava three-segmented. Malar sulcus present. Midlobe of mesoscutum with two pairs of setae. Notauli complete,

reaching the posterior margin of mesoscutum. Scutellum with lateral lines and two pairs of setae. Postmarginal vein equal to or shorter in length than stigmal vein. Submarginal vein with more than two setae. Forewings usually without fuscate markings. Pronotum with simple median carinae. Petiole wider than long.

Taxonomy. Cirrospilus is a cosmopolitan genus with at least 150 species (Noyes, 2003). Identification keys to New World species are not available. Zhu *et al*. (2002) provided a key to 20 Chinese species, including *C. variegatus* (Masi), which was reared from coffee leafminer in Peru (De Santis 1983a).

Biology and Host Records. Cirrospilus hosts include mostly Coleoptera, Diptera, and Lepidoptera living within plant tissues, and *Cirrospilus* species act as primary or secondary ectoparasitoids (LaSalle & Parella, 2005). Notley (1948) recorded *Cirrospilus cinctiventris* (Ferrière) as both a primary and secondary parasitoid on *Leucoptera* species in Africa, and *C. variegatus* (Masi) as a primary parasitoid that also showed a host feeding habit. At least ten specimens from Chiapas were reared as ectoparasitoids from coffee leafminer larvae (table 4.3). Unidentified *Cirrospilus* species from *L. coffeella* were recorded from Brazil (Villacorta, 1975; Reis *et al*., 1976; Parra *et al*., 1977; Souza, 1979; Zucchi *et al*., 1979; Souza *et al*., 1980; Villacorta, 1980; Guimaraes, 1983; Tozatti & Gravena, 1988; Aviles & Cure, 1990; Pollack, 1992; Mendoza, 1995), Colombia (Flórez & Hernández, 1982), Cuba (Konnorova, 1987; Carracedo & Zorrilla, 1989), Ecuador (Mendoza, 1995), Mexico (Aranda-Delgado, 1986), and Peru (Enriquez *et al*., 1975). *Cirrospilus variegatus* (Masi) was reported from Peru by De Santis (1983a).

***Closterocerus* Westwood, 1833**

Diagnosis (Schauff, 1991; Hansson, 1994; Burks, 2003). Head with frontofacial suture V-shaped, antennal scrobes not joining before meeting frontal suture, and extending below toruli ventrally. Clypeus barely defined by sutures. Funicle two-segmented, and clava with three segments; all antennal segments distinctly flattened. Malar sulcus absent. Midlobe of mesoscutum with one to four pairs of setae; anterior portion of notauli curving sharply laterad. Scutellum with one pair of setae. Forewings usually with one to three transverse fuscate bands. Postmarginal vein shorter than stigmal vein. Submarginal vein with two setae. Pronotum small, transverse, smooth, and shiny. Petiole wider than long.

Taxonomy. Closterocerus is a large genus, and Hansson (1994) provided a key to 21 species from the Nearctic region. The status and limits of this genus were unclear until Hansson's (1994) rewiew. Following publication of Hansson (1994), some species described as *Closterocerus* were placed in *Chrysonotomyia, Cirrospilus,* or *Neochrysocharis*. However, Noyes (2003) listed *Neochrysocharis* species under this genus. Keys for Neotropical species are not available, and Hansson (1994) did not include all the species known from coffee leafminer.

Biology and Host Records. Species in this genus are primary endoparasitoids of Coleoptera, Diptera, and Lepidoptera, although some secondary parasitoids have been recorded (Hansson, 1994). Notley (1948) reared *C. africanus* Waterston as a secondary parasitoid on *Leucoptera* sp. from Africa. Three species, all endoparasitic on coffee leafminer larvae, were collected in Chiapas, *Closterocerus cinctipennis* Ashmead and

two morphospecies not identifiable to species with the available keys (table 4.3). Unidentified *Closterocerus* species were recorded from Brazil (Avilés, 1991; Mendoza, 1995), Colombia (Le Pelley, 1968; as *Achrysocharis*), Cuba (Carracedo & Zorrilla, 1989), Mexico (Aranda-Delgado, 1986), and Puerto Rico (Wolcott, 1936 as *C.* nr. *cinctipennis*); *Closterocerus cinctipennis* Ashmead from Cuba **(**Le Pelley, 1968; Alayo & Hernández, 1978; De Santis, 1979; Mendoza, 1995), and Puerto Rico (Wolcott, 1936). *Closterocerus coffeellae* Ihering from Brazil (Le Pelley, 1968; Villacorta, 1975; Parra *et al*., 1977; Souza, 1979; Zucchi *et al*., 1979; Villacorta, 1980; Souza *et al*., 1980; D'Antonio *et al*., 1981; Paulini *et al*., 1983, Gravena, 1983; Guimarais, 1983; Tozatti & Gravena, 1988; Avilés, 1991; Mendoza, 1995), Cuba (De Santis, 1980), and Colombia (Le Pelley, 1968; Flórez & Hernández, 1982; Mendoza, 1995); *Closterocerus flavicinctus* De Santis from Brazil (De Santis, 1983b; Gravena, 1983); *Closterocerus leucopus* Ashmead from Puerto Rico (Wolcott, 1936, 1947; Le Pelley, 1968; De Santis, 1979; 1986; Mendoza, 1995), and San Vicente (De Santis, 1979). *Closterocerus lividus* (Ashmead) was recorded from Colombia, Puerto Rico, San Vicente, Grenada, and Venezuela by De Santis, (1979; 1983b) as *Chrysonotomyia* (*Achrysocharella*) *livida*, and from Colombia (Cárdenas, 1991; Mendoza, 1995 as *Chrysocharis livida*), Puerto Rico (Wolcott, 1936, 1947, as *Chrysocharis lividus;* Le Pelley, 1968; Mendoza, 1995), and Venezuela (Le Pelley, 1968; Mendoza, 1995) as a coffee leafminer parasitoid.

***Derostenus* Westwood, 1833**

Diagnosis (Hansson, 1986; Burks, 2003). Head with frontal suture reduced or absent; longitudinal grooves along with scrobal depressions forming a notorious

depression at the center of the face. Ocellar triangle encircled by a groove; posteromedial vertex with a cavity, occipital margin with two strong carinae; temples densely pubescent. Flagellum with three or four funicular, and two claval segments. Mesoscutum midlobe with two pairs of setae, notauli incomplete. Scutellum with one pair of setae. Forewings with two setae in the submarginal vein. Propodeum smooth or with median carina. Petiole at least twice as long as wide.

Taxonomy. This is a small, cosmopolitan genus with at least 12 species (Noyes, 2003). Hansson (1986) revised the Asian, European, and North American species. Many species were described under *Derostenus*, and later moved to other genera. Therefore, it is possible that some records on coffee leafminer are incorrect. For example, *Neochrysocharis formosa* (Westwood) was reported from coffee leafminer in Puerto Rico by Wolcott (1947) as *Derostenus* nr. *fullawayi*, and Le Pelley (1968) reported *Platocharis coffeae* as *Derostenus coffeae* from Puerto Rico.

Biology and Host Records. *Derostenus* are primary endoparasitoids of Diptera and Lepidoptera (Hansson, 1986; Schauff, 1991). Notley (1948) reared some specimens as primary parasitoids on coffee leafminers from Africa. Records on coffee leafminer include *Derostenus* sp. from Puerto Rico (Mendoza, 1995), though it is likely that these records correspond to *Neochrysocharis*. Corresponding material could not be examined, so it is unclear whether this genus should remain associated to coffee leafminer.

***Elachertus* Spinola, 1811**

Diagnosis (Zhu & Huang, 2001; Burks, 2003). Frontal suture absent. Antenna with four funicular and two claval segments. Lateral surface of scape with area of

sensory pits. Midlobe of mesothorax with three or more setae; notauli complete. Scutellum with two pairs of setae; usually with longitudinal sublateral grooves. Forewings with three or more setae in the submarginal vein, postmarginal vein longer than stigmal vein. Propodeum with median carina present, but without plicae or costula. Petiole wider than long.

Taxonomy. *Elachertus* is a cosmopolitan genus that includes at least 122 species (Noyes, 2003). Schauff (1985) provided a key to six Nearctic species, and Zhu & Huang (2001) a key to 23 Chinese species.

Biology and Host Records. Most species in this genus are primary ectoparasitoids of Coleoptera, Diptera, and Lepidoptera larvae (Schauff, 1985; Fidgen & Eveleigh, 1998), but some are secondary parasitoids (Bouček, 1988). Two unidentified species from Chiapas were reared as ectoparasitoids of coffee leafminer larvae (table 4.3). *Elachertus* sp. was recorded on coffee leafminer in Brazil (Aviles & Cure, 1990; Mendoza, 1995), Cuba (Le Pelley, 1968; Alayo & Hernández, 1978; Carracedo & Zorrilla, 1989), Mexico (Aranda-Delgado, 1986), and Puerto Rico (as *Cirrospiloideus* sp. in Wolcott, 1936, 1947; Le Pelley, 1968, as *Elachertus* sp; Gallardo, 1988; Mendoza, 1995).

***Euderus* Haliday, 1844**

Diagnosis (Yoshimoto, 1971; Burks, 2003). Head transverse, and eyes prominent. Antennae with two anelli, and four funicular and three claval segments. Midlobe of mesoscutum with more than three setae dorsally; notauli complete. Scutellum with two pairs of setae, and without grooves. Forewings hyaline, usually with

a bare area at their base; submarginal vein with more than three setae on its dorsal surface; two or three rows of setae radiating from the stigmal vein; length of postmarginal vein less than twice the length of stigmal vein. Propodeum with median carinae. Petiole wider than long.

Taxonomy. This genus includes at least 76 species (Noyes, 2003). Yoshimoto (1971) provided a key to 15 species from North America.

Biology and Host Records. Species in this genus are usually primary endoparasitoids of concealed hosts, but some species are secondary parasitoids (Yoshimoto, 1971). *Euderus lividus* (Ashmead) was reared as a primary ectoparasitoid by John & Sebastian (2002) on *Melanogromyza obtusa* (Malloch). Records on coffee leafminer include *Euderus* sp., and *E. lividus* from Puerto Rico (Herting, 1975).

***Eulophus* Geoffroy, 1762**

Diagnosis (Zhu & Huang, 2002; Burks, 2003). Mandibles reduced, not meeting medially. Antenna with three or four funicular and two or three claval segments; males of some species with long branches in the funicle. Notauli incomplete. Scutellum with paired setae, and without median grooves. Basitarsus distinctly shorter than second tarsal segment. Forewings hyaline, submarginal vein with more than three setae; postmarginal vein longer than the stigmal vein. Propodeum with median carinae and plicae.

Taxonomy. This genus includes at least 77 species (Noyes, 2003). Zhu & Huang (2002) reviewed eight Chinese species. Keys for New World species are not available.

Biology and Host Records. Eulophus species are gregarious ectoparasitoids (Marris & Edwards, 1995; Richards & Edwards, 2000), mainly of Lepidoptera, but also

of Coleoptera, Diptera, and Hymenoptera (Noyes, 2003). Records on coffee leafminer include *Eulophus* sp. from Brazil (Thompson, 1955; Le Pelley, 1968; Mendoza, 1995), Colombia (Le Pelley, 1968; Herting, 1975; Mendoza, 1995), and Cuba (Mendoza, 1995), and *E. cemiostomatis* Mann from Brazil (Mann, 1872; De Santis, 1980).

***Horismenus* Walker, 1843**

Diagnosis (Burks, 1971; Schauff, 1991; Burks, 2003). Head with frontal grooves V-shaped. Female antennae with three, and male antennae with four funicular segments. Midlobe of mesoscutum with two pairs of setae. Scutellum with one pair of setae and a median longitudinal groove. Forewings hyaline; submarginal vein with two dorsal setae; stigmal vein without distinct petiole; postmarginal vein reduced. Propodeum with raised smooth area bounded laterally by depressed, usually sculptured areas. Petiole twice as long as wide, and reticulate.

Taxonomy. This is a cosmopolitan genus that includes at least 62 species (Noyes, 2003). Burks (1971) provided a key to Nearctic species, and Hansson *et al.* (2004) to Mexican species associated with Bruchidae (Coleoptera).

Biology and Host Records. Most species are larval and/or pupal parasitoids of Coleoptera, Diptera, and Lepidoptera (Schauff, 1991; Hansson *et al.,* 2004), though some species are secondary parasitoids on Braconidae and Chalcidoidea species (Schauff, 1991). Two unidentified species (13 specimens) were reared from coffee leafminer larvae from Chiapas (table 4.3). Records on coffee leafminer include *Horismenus* sp. from Brazil (Parra *et al*., 1977; Gonçalvez, 1978; Souza, 1979), Cuba (Konnorova, 1987; Carracedo & Zorrilla, 1989), Mexico (Aranda-Delgado, 1986, as

Psephenivorus sp.), and Puerto Rico (Gallardo, 1988); *Horismenus aeneicollis* Ashmead from Brazil (LePelley, 1968; Villacorta, 1975; Herting, 1975; Parra *et al*., 1977; Zucchi *et al*., 1979; De Santis, 1980; Villacorta, 1980; D'Antonio *et al*., 1981; Paulini *et al*., 1983; Aviles *et al.,* 1985; Mendoza, 1995); and, *Horismenus cupreus* (Ashmead) from Brazil (Reis *et al*., 1976; Aviles & Cure, 1990; Mendoza, 1995, as *Horisaenus cupreus*), Colombia (Cárdenas, 1991; Flórez & Hernández, 1982; Mendoza, 1995, as *Horisaenus cupreus*), Cuba (LePelley, 1968; Alayo & Hernández, 1978; De Santis, 1979; Mendoza, 1995, as *Horisaenus cupreus*), Ecuador (Mendoza, 1995), Grenada (De Santis, 1979), Guatemala (Aranda-Delgado, 1986), Puerto Rico (Wolcott, 1936; LePelley, 1968; De Santis, 1979; Mendoza, 1995, as *Horisaenus cupreus*), and San Vicente (De Santis, 1979).

***Microlycus* Thomson, 1878**

Diagnosis (Kerrich, 1969; Burks, 2003). Antenna with three or four funicular, and two or three claval segments; males with three funicular segments with short branches. Toruli below eye margins. Lateral ocelli near eye margin. Midlobe of mesoscutum with numerous, irregularly distributed setae; notauli incomplete. Scutellum with two pairs of setae and without grooves. Postmarginal vein of forewings less than 1.7× length of stigmal vein. Propodeum with weak medial carinae.

Taxonomy. This genus includes at least 10 species (Noyes, 2003), mostly from the Old World. Keys to species are not available.

Biology and Host Records. Biology is known from only a few species (Noyes, 2003). Most species are parasitoids of Lepidoptera larvae. Recorded on coffee leafminer

only from Peru, as *Microlygus* (*sic*) sp. (Enriquez *et al.,* 1976); this is the only record of *Microlycus* available from the New World, thus it is questionable whether this genus is truly associated with coffee leafminer.

***Miotropis* Thomson, 1878**

Diagnosis (Burks, 2003). Antenna with funicle with four and clava with two segments. Notauli complete. Midlobe of mesoscutum with at least two pairs of setae. Scutellum with two pairs of setae, submedial grooves incomplete or absent, and straight when present. Forewing with postmarginal vein longer than stigmal vein. Propodeum with medial carinae, but without plicae.

Taxonomy. This genus includes at least 14 species (Noyes, 2003). A number of species initially described under *Cirrospiloideus* Ashmead now belong to this genus (Schauff & LaSalle, 1993). Keys to the species of this genus are not available.

Biology and Host Records. Species of this genus are primary ectoparasitoids of Lepidoptera. Previous records on coffee leafminer are limited to *Miotropis* sp. from Cuba (Carracedo & Zorrilla, 1989). Three specimens, identified as *Miotropis* Thompson using the key of Schauff *et al.* (1997), were reared from coffee leafminer larvae in Chiapas, Mexico, and represent a new record for coffee leafminer from Mexico (table 4.2).

***Neochrysocharis* Kurdjumov, 1912**

Diagnosis (Hansson, 1995; Schauff *et al.,* 1997, 1998). Head with frontofacial suture V-shaped; clypeus delimited by sutures. Antennae with three small and discoidal annelli, distal annellus sometimes large; funicle two-segmented, and clava three-

segmented. Notauli absent or poorly defined. Scutellum with one pair of setae, and without grooves. Forewings hyaline, without line of setae extending distally from stigmal vein; submarginal vein with two setae dorsally; postmarginal vein shorter than stigmal vein. Propodeum short, transverse, and usually smooth and shiny, without median carinae or plicae. Petiole wider than long.

Taxonomy. *Neochrysocharis* is a cosmopolitan genus with at least 18 Nearctic species (Schauff, 1997), and at least 48 species worldwide (Noyes, 1998). This genus has a complicated history. Some species were originally placed in *Closterocerus*, *Chrysonotomyia*, or *Chrysocharis* (Hansson, 1990, 1995). Bouček (1988) established *Neochrysocharis* as a synonym of *Chrysonotomyia*. Burks (2002) included this genus in his key, but later removed and incorporated it into *Closterocerus* (Burks, 2003). Schauff (1991) placed the species in a key to *Closterocerus*, and subsequently, in a key to Nearctic Eulophidae, he included *Neochrysocharis* as a valid genus (Schauff *et al.,* 1997). Hansson (1990, 1994, 1995, 1997) treated *Neochrysocharis* as a valid genus, while Noyes (2003) placed all the species under C*losterocerus*. Hansson (1995) was followed herein, so *Neochrysocharis* is treated as a valid genus. Keys to species of this genus are available in Hansson (1995) for Nearctic species, Hansson (1990) for Palearctic species, and Hansson (1997) for Mexican species.

Biology and Host Records. *Neochrysocharis* includes endoparasitoids of larvae of leafmining and stemboring Lepidoptera, Coleoptera, and Diptera, but some species are secondary parasitoids on Hymenoptera (Patel & Schuster, 1992; Hansson, 1995). At least five species were collected in Chiapas, Mexico, including two species that could

not be identified to species level with the available keys (table 4.3). *Neochrysocharis arvensis* Graham and *N. chalybea* Hansson were new records for coffee leafminer, and *N. formosa* (Westwood) was a new record for Mexico. Specimens from Chiapas were reared as ectoparasitoids of coffee leafminer larvae (table 4.1). Previous records on coffee leafminer include *N. formosa* (Westwood) from Puerto Rico (Wolcott, 1947, as *Derastenus* nr. *fullawayi* Crawford), and *N. arata* (Walker) from Peru (Enriquez *et al*., 1975, as *N. immaculata* Kurdjumov).

***Pediobius* Walker, 1846**

Diagnosis (Schauff, 1991; Schauff *et al*., 1998; Burks, 2003). Frontofacial suture V-shaped, scrobal grooves reaching transverse grooves independently. Antenna with three funicular and two claval segments. Pronotal collar with carinae. Midlobe of mesoscutum with two pairs of setae. Scutellum with one pair of setae, and lacking grooves. Forewings hyaline; submarginal vein with two setae; postmarginal vein shorter than stigmal vein, stigmal vein without petiole. Propodeum with a pair of medial carinae, which diverge posteriorly; lateral plica present. Petiole conspicuous and with ventrally projecting teeth.

Taxonomy. With at least 220 species described, *Pediobius* is one of the largest genera in Eulophidae (Noyes, 2003). Peck (1985) gave a key to 32 Nearctic species, and Hansson (2002) gave a key to 25 species of Costa Rica and adjacent countries.

Biology and Host Records. This genus includes primary and secondary parasitoids of a variety of taxa, including Lepidoptera, Diptera, Hymenoptera, Thysanoptera, and arachnids (Dawah *et al*., 2002; Hansson, 2002). *Pediobius coffeicola*

Ferrière was reared as a primary endoparasitoid from *Leucoptera* species from Africa, and as a secondary parasitoid on *Eulophus* and *Cirrospilus* species (Notley, 1948). Neotropical records on *L. coffeella* include only *Pediobius* sp. from Peru (Enriquez *et al*., 1975).

***Platocharis* Kerrich, 1969**

Diagnosis (Kerrich, 1969). Antenna with three funicular and two claval segments. Scutellum with one pair of setae and without median groove, but with very fine lateral grooves present. Forewings hyaline; submarginal vein with two setae; postmarginal vein longer than stigmal vein. Propodeum with carinae bordering median area strongly angled, lateral plicae present.

Taxonomy. This genus contains one species (Noyes, 2003). Ferrière (1936) described *Platocharis coffeae* (Ferrière) as *Derostenus coffeae* Ferrière from *Leucoptera meyricki* (Ghesquiere) collected in Africa. Subsequently, Kerrich (1969) placed this species in *Platocharis*.

Biology and Host Records. *Platocharis* was reared from coffee leafminer as a pupal parasitoid (Le Pelley, 1968). Noyes (2003) cited it as a parasitoid of *Leucoptera* spp., but not coffee leafminer. The only record on coffee leafminer was provided by Le Pelley (1968) from Puerto Rico, as *Derostenus coffeae*. Subsequent studies in Puerto Rico did not find this species (Gallardo, 1988), so it is unclear whether this genus should remain associated to coffee leafminer in the Neotropics.

***Pnigalio* Schrank, 1802**

Diagnosis (Schauff *et al*., 1998; Burks, 2003). Antennal flagellum usually with four funicular segments; males with first three basal segments branched. Notauli incomplete, or not reaching the scutellum. Scutellum without grooves. Forewing hyaline; submarginal vein with three or more setae dorsally; postmarginal vein longer than stigmal vein. Propodeum with medial carina, costulae, and plicae; median area smooth and shiny. Petiole longer than wide.

Taxonomy. Pnigalio is a cosmopolitan genus with at least 54 species (Noyes, 2003). Yoshimoto (1983) reviewed 17 species from North America, and provided an identification key.

Biology and Host Records. Species of *Pnigalio* are ectoparasitoids of concealed hosts in the orders Lepidoptera, Coleoptera, and Diptera (Yoshimoto, 1983; Oatman, 1985). At least three species were collected in Chiapas, Mexico; two could not be identified to species level with the available keys, while the other, *P. sarasolai* De Santis, was a new record for Mexico (table 4.2). Specimens from Chiapas were reared as ectoparasitoids on coffee leafminer larvae. Records on coffee leafminer include *Pnigalio* sp. from Colombia (Flórez & Hernández, 1982), Ecuador (Mendoza, 1995, as *Prigalio* sp.), El Salvador (Aranda-Delgado, 1986), and Guatemala (Campos *et al*., 1989); *Pnigalio* nr. *coloni* (Girault), and *Pnigalio elongatus* (Yoshimoto) from Mexico (Aranda-Delgado 1986); and, *Pnigalio sarasolai* De Santis from Colombia (De Santis 1983a, 1989; Cárdenas, 1991; Mendoza, 1995, as *Prigalio sarasolai*), and Guatemala (Mendoza, 1995, as *Prigalio sarasolai*).

***Proacrias* Ihering, 1914**

Diagnosis (Bouček 1977; Burks, 2003). Frontofacial suture V-shaped; scrobal grooves reaching transverse groove separately. Antenna with six segments, including one anellus and two funicular segments. Pronotum with carina. Midlobe of mesoscutum with two pairs of setae; notauli curving sharply laterally. Forewings with postmarginal vein short or longer than stigmal vein. Propodeum with modified median carina, either broadened and dorsally flattened or split posteriorly; plicae sometimes present.

Taxonomy. This genus includes at least five species (Noyes, 2003). Bouček (1977) provided a key to four Neotropical species in this genus.

Biology and Host Records. Species in this genus have been reared from lepidopteran and dipteran leafminer larvae. Ihering (1914) reared *Proacrias coffeae* Ihering as a secondary parasitoid on coffee leafminer from Brazil. Records on coffee leafminer include *Proacrias* sp. from Brazil (Reis *et al*., 1976; Souza, 1979; Souza *et al*., 1980; D'Antonio *et al*., 1981; Guimarais, 1983; Mendoza, 1995), Cuba (Konnorova, 1987; Carracedo & Zorrilla, 1989, as *Poacreas* sp.), and Venezuela (Rosales-Mondragón *et al*., 2003); and, *Proacrias coffeae* from Brazil [as *Neochrysocharis coffeae* (Ihering) in Ihering, 1914; Le Pelley, 1968; Herting, 1975; Villacorta, 1975; Parra *et al.,* 1977; Gallo *et al*., 1978; Gonçalvez, 1978; Zucchi *et al.,* 1979; De Santis, 1980; Villacorta, 1980, Gravena, 1983; Paulini *et al*., 1983; Tozatti & Gravena, 1988; Mendoza, 1995], Cuba (De Santis, 1979, as *N. coffeae*), Colombia (Le Pelley, 1968; Mendoza, 1995), and Puerto Rico (Wolcott, 1936).

***Tetrastichus* Haliday, 1844**

Diagnosis (Schauff *et al*., 1998). Antenna with three funicular segments in females, and four in males. Notauli complete; mesoscutum with a line of setae just mesal to notaulus; scutellum with two pairs of setae and two pairs of longitudinal lines. Propodeum with Y-shaped paraspiracular carina. Forewings with a single dorsal seta in submarginal vein; postmarginal vein reduced or absent.

Taxonomy. This is a large, cosmopolitan genus with at least 469 species (Noyes 2003). Burks (1943) provided a key to the North American species, most of which have now been placed in other genera, and Graham (1991) a key to European species.

Biology and Host Records. Hosts of this genus include insects in the orders Coleoptera, Diptera, and Lepidoptera, as well as spiders and mites (Noyes, 2003). Only generic records are available for coffee leafminer, and are from Brazil (Gallo *et al*., 1978; Zucchi *et al*., 1979), Colombia (Flórez & Hernández, 1982; Mendoza 1995), Ecuador (Mendoza, 1995), and Puerto Rico (Wolcott, 1947; Le Pelley, 1968). Specimens from Veracruz, Mexico, reported by Aranda-Delgado (1986) do not correspond to *Tetrastichus*, but to *Aprostocetus*. The same may be true for other records on *Tetrastichus* from other Neotropical countries (table 4.2).

***Zagrammosoma* Ashmead, 1904**

Diagnosis (Schauff *et al*., 1998; Burks, 2003). Head vaulted, with vertex distinctly higher than eyes. Antenna with funicle two segmented. Notauli present, ending in anterior half of strongly advanced axillae. Scutellum with lateral grooves. Forewings

with fuscate areas; submarginal vein with three or more setae dorsally, postmarginal vein subequal in length to stigmal vein. Propodeum without plicae, and carina simple.

Taxonomy. This genus includes at least 13 species (Noyes, 2003), mainly from the New World. Gordh (1978) gave a key to nine Nearctic species.

Biology and Host Records. Species in this genus are solitary ectoparasitoids of Lepidoptera and Diptera leafminers (Gordh, 1978; Maier, 1988), but some species are facultative secondary parasitoids (Gordh, 1978). Two species were collected in Chiapas, Mexico, *Z. lineaticeps* and *Z. multilineatum* (table 4.3). Records on coffee leafminer include *Zagrammosoma* sp. from Brazil (Gravena, 1983), Colombia (Cárdenas, 1991; Mendoza, 1995), Cuba (Carracedo & Zorrilla, 1989), Ecuador (Mendoza, 1995), Guatemala (Campos *et al*., 1989; Mendoza, 1995), Mexico (Aranda-Delgado, 1986), Peru (Pollack, 1992), and Puerto Rico (Wolcott 1947; Gallardo 1988); *Zagrammosoma lineaticeps* (Girault) from "Central America," and West Indies (Burks, 1979, as *Mirzagrammosoma lineaticeps;* De Santis, 1979), and Mexico (De Santis, 1979); *Zagrammosoma multilineatum* Ashmead from Brazil (Mendoza, 1995, as *Cirrospilus multilineatus*), Colombia (De Santis 1983a, as *Z. zebralineatum;* Cárdenas, 1991, as *C. zebralineatum;* Flórez & Hernández, 1992, as *C. multilineatus;* Mendoza, 1995), Cuba (Le Pelley, 1968, as *C. multilineatus;* De Santis, 1979; Carracedo & Zorrilla, 1989; Flórez & Hernández, 1992, as *C. multilineatus*), Jamaica (Le Pelley 1968, as *C. multilineatus*, De Santis 1979), Mexico (Aranda-Delgado, 1986), Puerto Rico (Wolcott 1936, as *Z. multilineata;* Le Pelley, 1968, as *C. multilineatus*; De Santis, 1979*;* Flórez & Hernández, 1992, as *C. multilineatus*), and Venezuela (Le Pelley, 1968, as *C.*

multilineatus; De Santis, 1979, as *C. multilineatus;* Flórez & Hernández, 1992, as *C. multilineatus*); and, *Zagrammosoma seini* Wolcott from Puerto Rico (Wolcott, 1936; De Santis, 1979).

Check list of Braconidae coffee leafminer parasitoids

***Allobracon* Gahan, 1915**

Diagnosis (Wharton, 1993; Mitio-Shimbori, 2005). Ventral margin of clypeus and dorsal surface of mandibles forming an ovoid cavity (cyclostome condition). Occipital carina absent dorsally and laterally. Epicnemial carina absent. Forewings with r-m vein present, RS vein reaching wing margin as a tubular vein. Propodeum without median areola, but with median longitudinal carina. Petiole partly desclerotized posteriorly, usually bearing a median carina. Metasoma membranous.

Taxonomy. This genus is restricted to the New World, and has 12 described species (Penteado-Dias & van Achterberg, 2004). Mitio-Shimbori (2005) reported 12 new species from Brazil, though at this time descriptions have not been formally published. Penteado-Dias and van Achterberg (2004) provided a key to the described Neotropical species.

Biology and Host Records. Biology is poorly known, but some species have been reared from Coleoptera and Lepidoptera leafminer larvae. Forty five specimens of *Allobracon* sp. were collected as endoparasitoids on coffee leafminer larvae from Chiapas in the present study (table 4.3), though none correspond to known species, and likely belong to an undescribed species (R. Wharton, Texas A&M University, personal

communication, 2006). Previous records on coffee leafminer include *Allobracon* sp. from Honduras (Trejo-Sosa, 2004), and Colombia and Mexico (Aranda-Delgado, 1986, as *Leurinion* sp.); and, *A. primus* (Muesebeck) from Colombia (Flórez & Hernández, 1989, as *Leurinion* sp.? *primum*).

***Apanteles* Foerster, 1862**

Diagnosis (Whitfield, 1997a). Ventral margin of clypeus and dorsal surface of mandibles not forming an ovoid cavity (non-cyclostome condition). Occipital carina absent. Antenna with 18 segments. Forewings with RS vein not reaching wing margin as a tubular vein; r-m vein absent. Propodeum without median longitudinal carina. Hypopygium almost always with large medial membranous region.

Taxonomy. Apanteles is a cosmopolitan genus that probably includes more than 1,000 New World species (Whitfield, 1997a). Mason (1981) reviewed the genus, but a key to Neotropical species is not available.

Biology and Host Records. All species within this genus are parasitoids of Lepidoptera. *Apanteles bordagei* Giard was reared as a primary endoparasitoid of *Leucoptera* species in Africa (Notley, 1948). *Apanteles* sp. was reported in association with coffee leafminer in Guatemala by Aranda-Delgado (1986), though this record needs confirmation.

***Bracon* Fabricius, 1804**

Diagnosis (Quicke, 1997). Head has cyclostome condition. Occipital carina absent. Antenna with more than 20 flagellomeres in most species. Epicnemial carina

absent. Forewings with RS vein reaching wing margin as a tubular vein; r-m vein present. M+CU vein of hind wing less than half as long as 1M vein.

Taxonomy. This is a large, cosmopolitan genus, with more than 500 described, and thousands of undescribed species (CAB International, 2000). Quicke (1997) indicated that Neotropical species are virtually impossible to identify using available keys.

Biology and Host Records. Most species are parasitoids of Lepidoptera, Coleoptera, and Diptera larvae (Quicke, 1997). Records on coffee leafminer include *Bracon* sp. from Guatemala (Aranda-Delgado, 1986; Campos *et al*., 1989), and Puerto Rico (Wolcott, 1947, as *Microbracon* sp.).

***Mirax* Haliday, 1833**

Diagnosis (Whitfield, 1997b). Head does not have cyclostome condition. Occipital carina absent. Antenna with 12 flagellomeres. Forewings with reduced venation. R1, 3RS, and r-m veins completely absent. Second metasomal tergum with Y-shaped sclerite.

Taxonomy. This is a small genus that includes approximately 20 species in the Holarctic region, many of them undescribed (Whitfield & Wagner, 1991). Its taxonomy is poorly known, and while a high diversity was suggested for the tropics ,the region has not been explored (Whitfield, 1997b). Muesebeck (1922) reviewed North American species under the subfamily Microgasterinae, though currently they are placed in Miracinae (Whitfield, 1997b).

Biology and Host Records. Species in this genus are primary endoparasitoids on Lepidoptera larvae, though adults emerge from the host cocoons (Whitfield, 1997b). Records on coffee leafminer include *Mirax* sp. from Brazil (Villacorta, 1975; Gonçalves *et al*., 1978; Zucchi *et al*., 1979; Souza *et al.,* 1980; Villacorta, 1980; Antique *et al*., 1981; Paulini *et al*., 1983; Filho & Guimaraes, 1984; Antique, 1986); *Mirax insularis* Muesebeck from Guadeloupe Island, Dominica, Brazil (Muesebeck, 1937; LePelley, 1968; Villacorta, 1975; Gallo *et al*., 1978; Aviles *et al.,* 1985; Aviles & Cure, 1990; Mendoza, 1995), and Puerto Rico (Gallardo, 1988); and, *Mirax striata* (Penteado-Dias) from Brazil (Penteado-Dias, 1999, as *Centistidea striata* Penteado-Dias).

***Orgilus* Haliday, 1833**

Diagnosis (van Achterberg, 1997). Head has non-cyclostome condition. Occipital carina present laterally. Antenna with more than 20 flagellomeres. Epicnemial carina present. Forewings with 3RS vein reaching wing margin as a tubular vein; r-m vein completely absent. Ovipositor longer than the length of the hind femur.

Taxonomy. Orgilus is a cosmopolitan genus with more than 120 species in the New World (van Achterberg, 1997), including at least 109 species from the Nearctic region (Nomina Insecta Nearctica, 1998). Muesebeck (1970) provided a key to North American species, and van Achterberg (1987) a key to species from some New World species groups.

Biology and Host Records. Most species are solitary endoparasitoids of lepidopteran larvae, including leafminers (van Achterberg, 1987). Records on coffee leafminer include *Orgilus* sp. from Brazil (LePelley, 1968); *Orgilus niger* Penteado-Dias

from Brazil (Penteado-Dias, 1999); and, *O. punctatus* (Beyr), as *Eubadizon punctatus* Beyr from Brazil (Gallo *et al.,* 1978; Gonçalves *et al*., 1978; Zucchi *et al*., 1979; Antique *et al*., 1981; Filho & Guimaraes, 1984; Antique, 1986).

***Stiropius* Cameron, 1911**

Diagnosis (van Achterberg, 1995). Head has cyclostome condition. Occipital carina present. Antenna consisting of 20 or more flagellomeres. Forewings with 3RS reaching wing margin as a tubular vein; r-m vein present. Hind wing 1M vein longer than M+CU vein. Terga 2 + 3 carapace-like. Metasomal tergum 2 with median longitudinal carina.

Taxonomy. Most species are Neotropical, but some Nearctic species are known (Whitfield, 1988). van Achterberg (1995) provided a key to 17 species from North and Central America. Penteado-Dias (1999) described one new species from Brazil, *S. reticulates* Penteado-Dias. Shaw (1997) noted that many species likely remain undescribed, especially in the Neotropical region.

Biology and Host Records. Biology is known from only a few species, which *Stiropius* are solitary endoparasitoids of lepidopteran leafminers of the families Lyonetiidae and Gracillaridae (Shaw, 1997). More than 100 specimens of *Stiropius letifer* (Mann) were collected in Chiapas in the present study, all as larval endoparasitoids, though the adults emerged from the coffee leafminer cocoon (table 4.3); *S. letifer* is a new record for coffee leafminer in Mexico. Prior records on coffee leafminer include *Stiropius* sp. from Brazil (Parra *et al.*, 1997), Honduras (Trejo-Sosa, 2004), and Nicaragua (Maes *et al*., 2006); *Stiropius letifer* (Mann) from Brazil

(Villacorta, 1975; Reis *et al*., 1976, as *Colastes letifer* Mann; Gonçalves *et al*., 1978; Zucchi *et al*., 1979; Souza *et al*., 1980; Villacorta, 1980; Antique *et al*., 1981; Paulini *et al*., 1983; Filho & Guimaraes, 1984; Aviles *et al.,* 1985; Antique, 1986; Whitfield, 1988, as *Viridipyge letifer* (Mann)*;* Aviles & Cure, 1990), Ecuador (Mendoza, 1995, as *Viridipyge letifer*), Honduras (Aranda-Delgado, 1986, as *Bacculatriplex letifer* (Mann)), and Nicaragua (Whitfield 1988, as *Viridipyge letifer*); and, *Stiropius reticulatus* from Brazil (Penteado-Dias, 1999).

Genera likely associated erroneously with coffee leafminer

Ceranisus Walker, 1841

Aranda-Delgado (1986) reported *Ceranisus* sp. from Veracruz, Mexico. Two specimens identified as *Ceranisus* sp. in Aranda-Delgado (1986) were examined in the present study and neither corresponded to *Ceranisus*. One specimen, which was in poor condition, did not have the typical head of *Ceranisus*; the other specimen ran to *Closterocerus* in the Schauff *et al*. (1997) key. Therefore, this genus was not included in the list of coffee leafminer parasitoids for the Neotropical region compiled in the present study.

Diaulomorpha Ashmead, 1900

Aranda-Delgado (1986) reported the only record for this genus on coffee leafminer from one specimen collected in Veracruz, Mexico. It was labeled as "nr. *Alophomorpha* sp.," which is a generic synonym of *Diaulomorpha* (Bouček, 1988). This specimen was examined, and despite its poor condition ran to *Elachertus* in the Bouček

(1988) key. Therefore, this genus was not included in the coffee leafminer parasitoid list for the Neotropical region presented in this study.

***Sympiesis* Förster, 1856**

Records on coffee leafminer include *Sympiesis* sp. from Mexico (Aranda-Delgado, 1986), and *Sympiesis comosus* Kerrich from Africa (Kerrich, 1969). One specimen collected by Aranda-Delgado (1986) in Veracruz, Mexico, was examined, and though in poor condition clearly did not correspond to *Sympiesis*, running to *Pnigalio* in the Schauff *et al*. (1997) and Burks (2003) keys. Therefore, Aranda-Delgado's (1986) report is considered an error, and *Sympiesis* was not included in the coffee leafminer parasitoid list for the Neotropical region compiled in the present study.

CHAPTER V

IMPACTS OF RAIN, TEMPERATURE, AND SHADE COVER ON COFFEE LEAFMINER *Leucoptera coffeella* (LEPIDOPTERA: LYONETIIDAE) POPULATION DYNAMICS AND NATURAL ENEMIES

Introduction

Auerbach *et al.* (1995) cited three major sources of leafminer mortality: vertical sources, including natural enemies and host-plant attributes, horizontal sources, such as intra- and inter-specific competition, and abiotic sources, including meteorological factors, such as wind, storms, frosts, heavy rain, moisture, and extreme temperatures. While abiotic factors are not agents of population regulation, nor explain long-term persistence of populations, they limit and cause fluctuations in population size (Hixon *et al*., 2002). Meteorological factors affect insect population dynamics in different ways, e.g., insect development times decrease with increasing temperature, and survival rates decrease with heavy rain, low temperatures, and low moisture (Hespenheide, 1991; Nestel *et al*., 1994; Tipping *et al*., 2005). Also, mating and oviposition behaviors are negatively affected by heavy rain, low temperature, and strong wind (Wang *et al*., 1997; Saethre & Hofsvang, 2002). Similarly, meteorological factors have important direct and indirect effects on insect natural enemies (Wang *et al*., 1997; Weisser *et al*., 1997; Stireman *et al*., 2005). Stireman *et al*. (2005) suggested that natural enemies may be affected by unpredictable climatic variation itself, and by unpredictable amplified variation in host dynamics itself caused by changes in weather conditions.

Seasonal variation in weather variables, such as rainfall and temperature maxima and minima, may be the most important overall causes of dramatic changes in insect abundance, especially in temperate ecosystems (Cornell & Hawkins, 1995). Although the climate of tropical rain forests is comparatively constant, e.g., monthly average temperatures vary minimally throughout the year in tropical environments, marked local variation in temperature and precipitation occurs because of changes in topography, and such variation may affect insect population dynamics, as recorded in coffee farms in Veracruz, Mexico by Nestel *et al*. (1994). Understanding the impacts of weather variables on pest population dynamics is important for managing pests generally, including pests of tropical crops.

Coffee leafminer [*Leucoptera coffeella* (Guérin-Ménèville) (Lepidoptera: Lyonetiidae)] is a monophagous lepidopteran on *Coffea* species that is becoming increasingly important as a pest of coffee in many New World countries, including Mexico (CAB International, 2000; Ruíz *et al*., 2004). Previous studies addressed in part the influence of weather variables on coffee leafminer population dynamics, and showed that high incidence of this pest was associated with low rainfall and high temperature (Reis *et al*., 1976; Gravena, 1983; Nestel *et al*., 1994; Costa-Conceiçao *et al*., 2005). Some authors suggested that rain was the main source of coffee leafminer adult and larval mortality in tropical areas, particularly during the rainy season (Villacorta, 1980; Campos *et al*., 1989). However, such suggestions were based on field studies assessing the proportions of mined leaves on coffee plants rather than on direct evaluation of mortality rates or sources. In addition, major outbreaks of coffee leafminer in the

Neotropics were associated with reductions of shade cover, and increasing use of pesticide in coffee farms (Guharay *et al.,* 2001; Monterrey *et al.,* 2001; Fragoso *et al*., 2002; Carvalho *et al*., 2005). Both factors were assumed to lead to reductions in natural enemy populations (Fragoso *et al.*, 2002), though this was not tested.

Shade cover affects micro-environmental conditions within coffee farms, which may thus impact coffee arthropod population dynamics (Moguel & Toledo, 1999; Haggar *et al*., 2001). Coffee leafminer outbreaks in Brazil were sporadic through ~1970 when most coffee was grown under shade conditions, but the intensity and frequency of outbreaks increased dramatically with the introduction of highly productive coffee varieties that required full-sun conditions and greater use of agrochemicals (Fragoso *et al*., 2002). Moreover, prior studies showed that shade cover within coffee farms moderated daily fluctuations in temperature, evapotranspiration, and total radiation, and increased soil moisture and temperature (Barradas & Fanjul, 1986). Haggar *et al*. (2001) recommended shade cover of 40-60% for Nicaraguan coffee farms in areas with rainfall below 1500 mm/year, and 20-40% for farm in areas with rainfall above 1500 mm/year to enhance production and facilitate pest management. Moguel and Toledo (1999) noted that elimination of shade cover in coffee farms led to a less stable physical environment, and decreased microorganism abundance and diversity. Thus, increases in coffee leafminer populations in farms where shade was reduced may be related to ensuing changes in micro-environmental conditions, which may directly or indirectly affect coffee leafminer (Guharay *et al*., 2001).

To date the effects of temperature, rainfall, and shade cover on the dynamics of coffee leafminer or its natural enemies have not been evaluated in detail, particularly in southern Mexico coffee farms. The present study assessed through field surveys and laboratory experiments the effects of temperature, rainfall, and shade cover on seasonal survival rate, distribution, and abundance of coffee leafminer and its natural enemies at two elevations in the Soconusco region of Chiapas, Mexico. Additionally, oviposition by coffee leafminer females in relation to time of day or night was assessed to uncover any circadian oviposition cycle.

Materials and methods

Coffee leafminers were reared at El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, Mexico on detached coffee leaves (*Coffea arabica* L.) as described in Reis *et al.* (2000). A culture, started with field collected leafminers, was maintained in a room with constant temperature (26 ± 1 ^{o}C), humidity close to saturation, and a natural photoperiod (~13L: 11D). Upon emergence, coffee leafminer adults were sexed and fed with small droplets of pure honey. Coffee leafminer adults were held in 4 l containers for 24 h to allow them to mate before using them in the experiments described below. All laboratory experiments were conducted at ECOSUR in a room with the climatic conditions described above.

Field studies were conducted in three coffee farms representing two elevations, low (480-550 m) and high (~960 m), in the municipality of Cacahoatán, Chiapas, Mexico. The Soconusco region, including Cacahoatán, comprises Pacific coastal

lowlands that reach the foothills of the Sierra Madre, and has higher moisture than interior regions of Chiapas. Low elevation coffee farms were represented by *El Encanto* (14° 59' 21'' N; 92° 09' 55'' W; 480 meters above sea level) and *La Gloria* (15° 00' 07'' N; 92 ° 09' 24'' W, 550 m), and high elevation farms by *Alpujarras* (15° 04' 24'' N; 92 ° 10' 11'' W, 960 m). Comparisons between high and low elevation farms relied on *Alpujarras* and *El Encanto* or *La Gloria* farms, as available, and are noted below. The main coffee varieties in these farms were *Catuai*, *Bourbón*, and *Typica*, but all studies involved plants of the *Catuai* variety. No insecticides were used on the farms during the study period. Historical weather data, 1961-1990 (Comisión Nacional del Agua, 2006), were used for rainfall and temperature comparisons between low and high elevation farms. Weather data from the Cacahoatán (station #7018, 14º 04' N, 92º 10' W, 350 m) were used to characterize low elevation farms, and from El Perú, Tapachula, Chiapas (station #7058, 15º 06' N, 92º 16' W, 800 m) to characterize high elevation farms. In addition, weather data [average daily, maxima, and minima temperatures, rainfall, and days with measurable rainfall (>0.1mm)] for some field studies (see below *Effects of shade cover…and natural enemies*), were obtained from a research station, Centro de Investigación y Desarrollo de la Caña de Azúcar, in Tuxtla Chico, Chiapas (14° 57' N, 92° 10' W, 366 m), and from rain gauges and maximum/minimum thermometers placed within coffee farms for the present study.

Throughout, data recorded as proportions (mined leaves, coffee leafminer survival, and death rates) were arcsine $\sqrt{x}$-transformed. Before analyses, all data were tested for conformity to requirements for parametric analysis. Nonparametric analyses

were conducted as appropriate. Untransformed data are presented in text, tables, and figures. All statistical analyses were performed using SPSS 12.0 for Windows and/or Statistix 8.0 (SPSS, 2005; Analytical Software, 2003), and are described in Zar (1996) and/or Sokal & Rohlf (1995).

Effects of weather variables on coffee leafminer seasonal phenology and natural enemies. The impacts of weather variables, i.e. monthly minimum and maximum temperatures, rainfall, days with measurable rainfall, and evaporation, on the seasonal phenology of coffee leafminer and its natural enemies were assessed through monthly surveys at one low elevation farm (*El Encanto*) from June 2003 to July 2004. Separately, the impacts of monthly minimum and maximum temperatures, and rainfall on coffee leafminer incidence and mortality sources were compared between one low elevation farm (*El Encanto*) and the high elevation farm through monthly surveys from March to June 2005. For both studies, 10 coffee plants each month were randomly selected, and the proportions of mined leaves per plant were assessed using nine branches per plant. In addition, 180 mined leaves were collected monthly from both localities in plastic bags and examined in the laboratory to assess coffee leafminer survival and mortality rates. The numbers of mines, coffee leafminer eggs, larvae, and pupae per each leaf were recorded, and stage-specific survival rates and mortality factors were assessed through dissection of mines under a dissecting microscope at 25× magnification (Carl Zeiss Inc., Thornwood, NY). Previous studies (Aranda-Delgado, 1986) and preliminary observations showed that larval and pupal coffee leafminer mortality could be assigned to one of three factors. 1) Death by parasitoid: indicated by

the presence of immature parasitoids (larvae, pupae, or their remains) inside coffee leafminer mines or pupae, parasitoid exit holes, or by presence of black ovipositor-caused scars on coffee leafminer larvae. 2) Death by predator: indicated by torn mines in which the number of larvae within the mine was smaller than the number of eggshells at the base of the mine, or by torn or empty coffee leafminer pupal remains in the absence of evidence of parasitoid activity. 3) Death by factors other than parasitoids or predators (hereafter FOPP): mortality was assigned to this factor when it could not be unambiguously assigned to either death by parasitoid or by predator.

Correlation analyses were conducted to uncover any association between monthly climatic variables (monthly minimum and maximum temperatures, rainfall, days with measurable rainfall, and evaporation) and monthly proportions of mined leaves per plant, and coffee leafminer mortality due to parasitoids, predators, or FOPP, or survivorship (= those not killed by parasitoids, predators, or FOPP) per 180 leaf-sample. In addition, comparisons of proportions of mined leaves, and coffee leafminer survival and mortality due to parasitism, predation, or FOPP between coffee plants at low and high elevation were made for each month between March and June, 2005, using *t*-tests.

Effects of shade cover on within-farm rainfall distribution, temperature, and coffee leafminer incidence and natural enemies. Field surveys and experiments were conducted at one low elevation farm (*La Gloria*) during April-July 2005. Thirty plots were randomly selected within the farm, each plot consisting of four plants from two contiguous rows forming a square.

Effects of shade cover on rainfall distribution and temperature. Canopy cover, defined as percentage of foliage cover obscuring open sky as viewed from the ground (Feldpausch *et al*., 2005), was used as a shade cover index. Canopy cover was assessed for each plot via analysis of digital photos with Gap Light Analyzer 2.0 software (Frazer *et al*., 1999). Four photos were taken at the center of each of the 30 plots at 150 cm above the ground with a digital camera equipped with a FC-E8 fisheye lens. Canopy cover data used for analyses were the average of the four photos taken at each of the 30 plots. The 30 plots included shade covers between 13.8 and 77.7%, with a mean of 50.2±15.3%. Rainfall was measured daily for 14 consecutive days at the center of each of the 30 plots using four 60-cm^3 rain gauges at 1 m above the ground; one pair of rain gauges was attached to a wooden rod, and pairs were separated by ~1.5 m from each other. Shelter from direct rainfall was predicted to be directly related to shade cover, and the ratio of the variance to the mean of rainfall among rain gauges per each plot (=variance/mean) was used as a rainfall dispersion index (~ shelter from direct rainfall), and for statistical analyses. A maximum/minimum thermometer was placed in the canopy of one of the four plants in each of the 30 plots, and maximum and minimum temperatures were recorded daily between 9:00 and 10:00 h. Shade cover was assessed at the beginning of the study (June 22, 2005), and temperatures and rainfall were recorded daily from 22 June to 5 July, 2005. Simple correlation analyses were conducted to uncover any relationship between shade cover and individual weather variables (daily, maximum and minimum temperatures, and rainfall dispersion index).

Effects of shade cover on coffee leafminer incidence. Eight branches, each with four pairs of undamaged leaves, were labeled on each of the four plants in the 30 plots. Thus, a total of 64 undamaged leaves per plant (= 1920 leaves on 30 plants) were labeled at the beginning of the study (April, 2005). The proportion of mined leaves per plant was evaluated one month later following a period of low rainfall (<100 mm/month) (May, 2005). Mined leaves included those having at least one fresh mine, defined as any mine with an active larva within, or a dead larva and green plant tissue within. A second set of observations was made four months after branches were initially labeled, which was following a period of high rainfall (> 400 mm/month) (August, 2005). Simple correlation analyses were conducted to uncover any relationships between shade and proportion of mined leaves under low (May, 2005) or high rainfall (August, 2005).

Effects of shade cover on coffee leafminer survival and mortality due to natural enemies. Coffee leafminer larval stage survival and mortality rates were assessed by establishing coffee leafminer cohorts on one plant in each of the 30 plots. One branch without mined leaves on each plant was selected, and the apical three pairs of leaves of each branch were enclosed in a 30 cm nylon mesh sleeve. Two pairs of coffee leafminer adults (24 h-old) were enclosed in each sleeve for 48 h; leafminer adults were removed after this time, and the number of eggs laid was recorded. Sleeves were left on the branch for one week to avoid egg predation and allow larvae to initiate mines, and then removed to expose leafminers to natural enemies during their development (~1 week). Leaves were harvested and taken to the laboratory to assess larval survival rates and mortality due to predation, parasitism, or FOPP, under a

dissecting microscope (see above *Effects of weather variables …*). Coffee leafminer egg and pupa survival rates, and mortality due predation, parasitism, or FOPP were assessed concurrently by exposing coffee leafminers from the laboratory colony to natural enemies on coffee plants. One plant was selected in each of the 30 plots, and five leaves with coffee leafminer eggs or pupae (<24 h old) were stapled to coffee leaves on the selected plants. Each of the five leaves contained 10-30 coffee leafminer eggs per leaf or 1-2 pupae per leaf. Leaves were collected after 1 week of exposure and taken to the laboratory to assess survival and mortality rates of coffee leafminer eggs and pupae.

Data were subjected to simple correlation analyses to uncover any relationships between shade cover and coffee leafminer survival, and stage-specific mortality rates (= proportions) due to parasitism, predation, or FOPP.

Rainfall and elevation effects on coffee leafminer survival and mortality factors. Stage-specific survival and mortality rates of coffee leafminer eggs, larvae, and pupae were assessed during a period of low rainfall, <100 mm/mo (April 27 – May 12, 2005) and high rainfall, >400 mm/mo (June 27 – July 12, 2005) at one low elevation farm (*La Gloria*) and the high elevation farm (*Alpujarras*).

Average shade cover in the low elevation farm was 66.7 ± 5.3% (range = 38.0 - 86.6%), and average maximum and minimum temperatures, total rainfall, and days with measurable rainfall (>0.1 mm) during the period of low rainfall were 32.3 ^{o}C, 18.0 ^{o}C, 80.1 mm, and 4 d, respectively. Corresponding data during the period of high rainfall were 29.0 ^{o}C, 20.5 ^{o}C, 413.0 mm, and 13 d. Average shade cover in the high elevation farm was 76.5 ± 4.5% (range = 44.8 - 91.3%). Average maximum and minimum

temperatures for the low rainfall period were 28.5 ^{o}C and 13.5 ^{o}C, and for the high rainfall period were 25.0 ^{o}C and 15.7 ^{o}C; total rainfall and days with measurable rainfall were not reported for the high elevation farm because of calibration problems with the rainfall gauge.

Ten coffee plants were randomly selected at each elevation on two dates, April 27 and June 27, and one coffee leafminer cohort was established on one branch of each plant using the methodology described above (see *Effects of shade cover… incidence and natural enemies*). Sleeves were removed from branches one week later (May 4, or July 3) and the number of eggs per branch was recorded. At the same time, 10 coffee leaves with coffee leafminer eggs (4-9/leaf) or pupae (1-2/leaf) were exposed by stapling them to coffee leaves on the selected plants. At least 50 eggs (range = 10-30/leaf), and 10 pupae (range =1-2/leaf) were placed on each plant. All coffee leafminer eggs, larvae, and pupae were exposed to mortality sources during one week, after which the corresponding leaves were taken to the laboratory and examined under a microscope to determine egg, larva, and pupa survivorship, and mortality rates due to parasitism, predation, or FOPP, per each plant.

The influence of elevation (low elevation = *La Gloria*; high elevation = *Alpujarras*) and rainfall (low, < 100 mm/mo, April-May; high, > 400 mm/mo, June-July) on coffee leafminer survival rate and the contributions to coffee leafminer mortality of parasitoids, predators, and FOPP at each of the 10 plants were compared using the Scheirer-Ray-Hare extension of the Kruskal-Wallis test, a two-way nonparametric

analysis of variance (Sokal & Rohlf, 1995). Survival and mortality rates per each of the 10 coffee plants were used as data for analyses.

Temperature effects on coffee leafminer oviposition rate. The effects of three constant temperatures, 20, 25 and 28 ^{o}C (all ± 1 °C), on coffee leafminer 48-h oviposition were assessed under laboratory conditions. One lab-reared coffee leafminer female was place inside a 0.5 l plastic cage with one male and one coffee leaf; a droplet of pure honey was provided as a source of food for the leafminers. The coffee leafminer female was allowed to lay eggs for 48 h, after which both leafminers were removed, and the number of eggs laid per leaf was recorded. Thirty-six pairs of coffee leafminers adults were monitored at each temperature. All leaves were from a single coffee plant and from the 2nd pair on a branch, and were held individually on a polyurethane sponge saturated with water inside the plastic cage. Kruskal-Wallis one-way nonparametric analysis of variance was used to determine if the mean number of eggs laid per female differed among temperatures. Means separation was by Tukey's HSD at alpha = 0.05.

Circadian cycle of coffee leafminer oviposition. The circadian cycle of coffee leafminer oviposition was assessed under laboratory conditions at constant temperature (26 ± 1 ^{o}C) using 24-h old coffee leafminers. One coffee leafminer mating pair was held inside a 0.5 l plastic cage with a coffee leaf using the methodology described above (see "*Temperature effects… leafminer rate*"). Leaves were replaced and the number of eggs per leaf recorded every 4 h between August 3 and August 7, 2005. Leaves were replaced at 03:00, 07:00, 11:00, 15:00, 19:00 and 23:00 h. Solar radiation was recorded at those hours in a weather station at ECOSUR (14º53'12'' N, 92º17'11'' W, 114 m; Vantage

Pro^{TM} Weather Station, Davis Instruments, Davis, California, USA). Sunrise during the experimental days occurred at ~6:53 h, and sunset at ~19:38 h, and two daily periods were established for comparisons, from sunset to sunrise (scotophase), and from sunrise to sunset (photophase). The relationship between total numbers of eggs per female in 4 d during each 4-h period and solar radiation was assessed using the Spearman Ranks test. In addition, Kruskal-Wallis one way nonparametric analyses of variance was conducted to compare coffee leafminer oviposition (eggs/female/4-h period) between the scotophase and the photophase.

Results

Historical weather data (1961-1990) showed that monthly rainfall patterns were similar at the low and high elevation weather stations (fig. 5.1a). Rainfall exceeded 4000 mm during the year, with the dry season between December and March (rainfall < 100 mm/mo), and the rainy season between April and November (> 200 mm/mo) (fig. 5.1a). Monthly mean temperatures were higher at low elevation (fig. 5.1b). Yearly average temperature at low elevation was 26.2 ± 0.2 ^{o}C, and the range was 25.4 – 27.2 ^{o}C; the highest maximum temperature (34.1 ^{o}C) occurred in April, and the lowest minima (~19 ^{o}C) in January and February. Yearly average temperature at high elevation was 22.6 ± 0.3 ^{o}C, and the range was 22.0 – 23 ^{o}C; the highest maximum temperatures (~28 ^{o}C) occurred in April and March, and the lowest minima (~16 ^{o}C) in January and February (fig. 5.1b).

Effects of weather variables on coffee leafminer seasonal phenology and natural enemies. The monthly proportion of mined leaves per plant at a low elevation farm (*El Encanto*) was significantly correlated with maximum and minimum temperatures, monthly rainfall, and days with measurable rainfall (table 5.1). The proportion mined leaves was >0.20 during the rainy season (June-November 2003 and May-July 2004), and the highest proportion (~0.49) was in June-July 2004 (fig. 5.2a). The proportion of mined leaves during the early part of the dry season was <0.10 (December 2003 to February 2004) and was ~0.24 at the end of the dry season (fig. 5.2a).

Coffee leafminer survival rate was positively correlated with maximum temperature ($r = 0.67$; $P = 0.01$) (table 5.1). Predation, parasitism, and mortality due to FOPP were not significantly correlated with any climatic variables that were evaluated (table 5.1). Coffee leafminer survival rate was lowest (<0.2) during the rainy season (July-November 2003) and early in the dry season (January 2004), when monthly maximum temperatures were 33-34 ^{o}C; and, was highest (>0.35) late in the dry season and early in the rainy season (February to June 2004) (fig. 5.3). The highest survival rate (~0.65) occurred during the dry season, in March 2004 (fig. 5.3). Predation rates were highest (>0.3) during the rainy season and early dry season (September 2003-January 2004, and June-July 2004), and were lowest (<0.1) from the end of the dry season to the beginning of the rainy season (March-May 2004) (fig. 5.3).

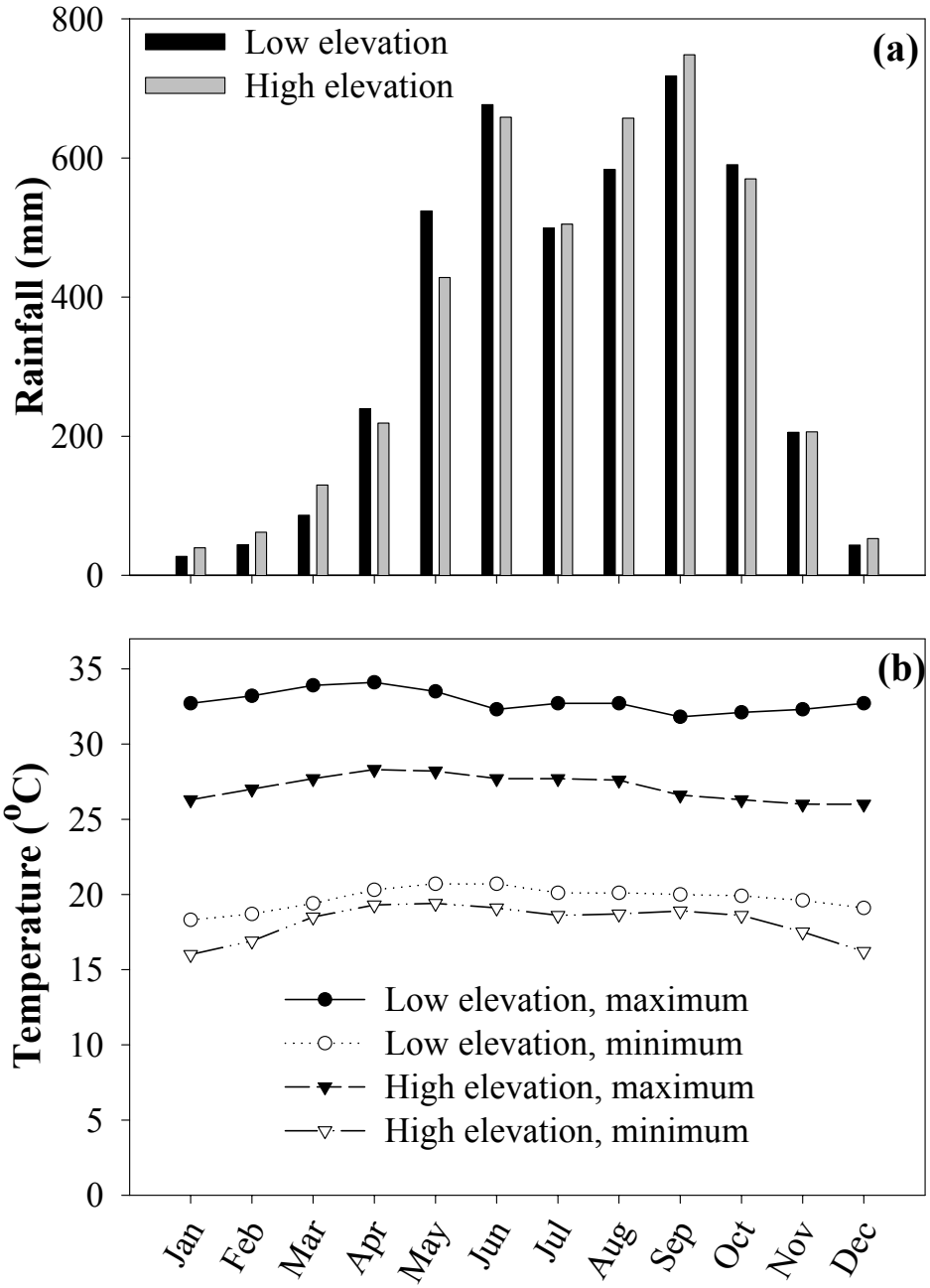

Fig. 5.1. Rainfall (a), and maximum and minimum monthly temperatures (b) at low (Cacahoatán, 14° 59' N, 92o 10' W, 350 m) and high elevations (El Perú, 15° 06' N, 92° 16' W, 800 m) in the Soconusco region of Chiapas, Mexico. Graphs show means of historical data, 1961 to 1990 (Comisión Nacional del Agua, 2006).

Table 5.1. Correlations between selected weather variables and proportion leaves mined by coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), and coffee leafminer survival rate and mortality factors (death due to predation, parasitism, or factors other than predation or parasitism), at a low elevation farm ~480 m (*El Encanto*), Cacahoatán, Chiapas, Mexico, June 2003 to July 2004.

Weather variables	Proportion of mined leaves[1]		Coffee leafminer survival and contributions of predation, parasitism, and other factors to total mortality[2]							
			Survival		Predation		Parasitism		Other	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
Maximum Temperature	-0.54	0.04	0.67	0.01	-0.51	0.06	-0.25	0.38	-0.38	0.18
Minimum Temperature	0.83	<0.01	-0.01	0.97	-0.18	0.53	-0.13	0.67	0.34	0.23
Monthly Rainfall	0.76	<0.01	-0.43	0.13	0.26	0.36	0.06	0.83	0.34	0.23
Days with measurable rainfall	0.85	<0.01	-0.33	0.24	0.21	0.46	0.01	0.99	0.30	0.30

[1] Estimated from 10 plants/month (9 branches/plant)

[2] Estimated from 180 leaves/month

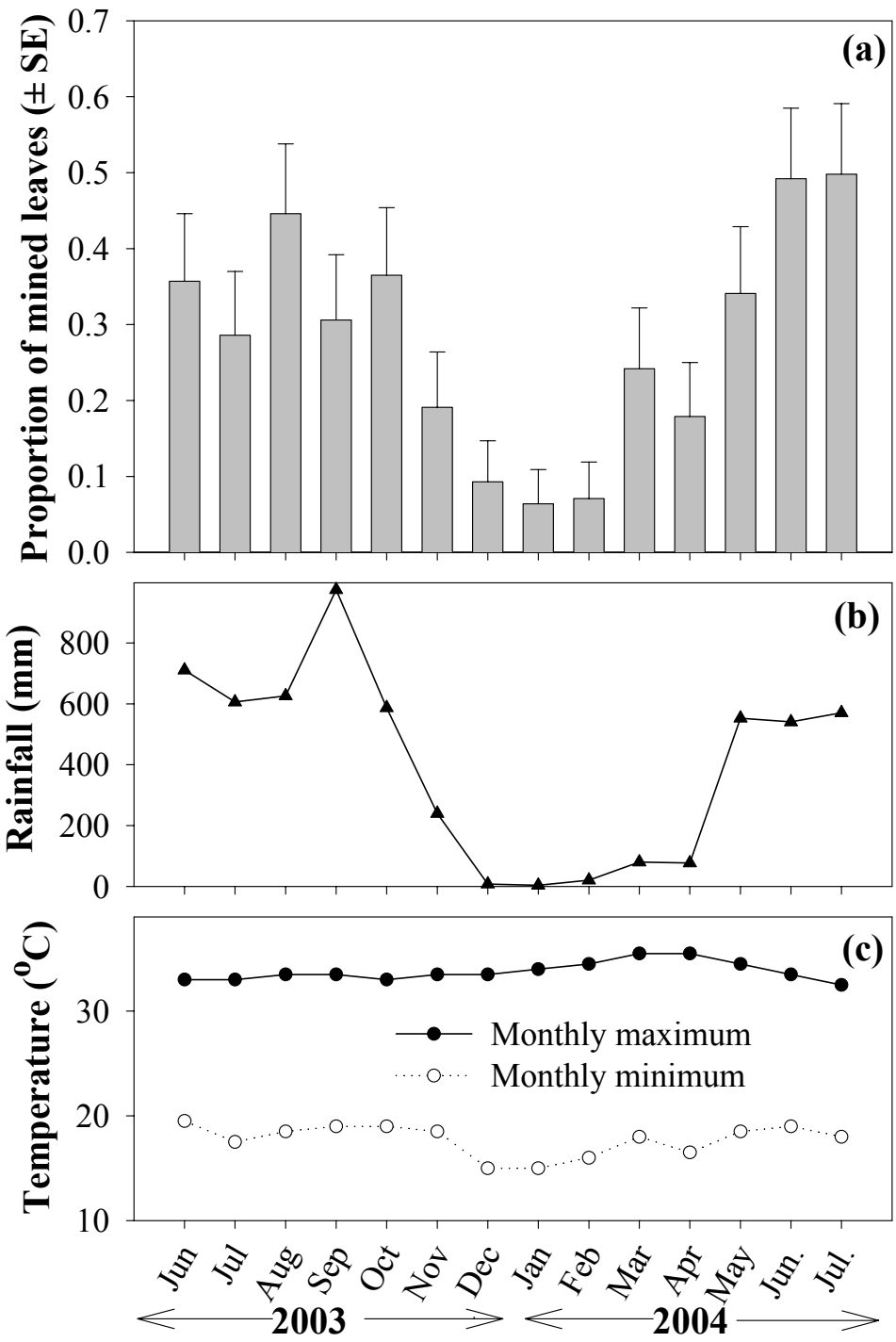

Fig. 5.2. (a) Proportion leaves mined by coffee leafminer, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) (± SE), (b) rainfall (mm), and (c) maximum and minimum monthly temperatures (^{o}C) at a low elevation coffee farm (*El Encanto*, 480 m), Cacahoatán, Chiapas, Mexico, 2003-2004.

The proportion of mined leaves during March to June 2005 was higher at the low versus high elevation farm, except in April when the difference was not significant (fig. 5.4). The proportion of mined leaves between March and June 2005 was not significantly correlated with maximum or minimum temperatures or monthly rainfall at either low or high elevations (table 5.2).

Coffee leafminer survival rate did not differ, except in June, between low and high elevation coffee farms (fig. 5.5a), and was negatively correlated with minimum temperature and rainfall, and positively with maximum temperature at the low elevation farm, but only positively with maximum temperature at the high elevation farm (table 5.2). Predation rates increased between March and June, but did not differ between elevations (fig. 5.5b). Predation rates were positively correlated with minimum temperatures and rainfall, and negatively with maximum temperatures at the low elevation farm, but only negatively with maximum temperature at the high elevation farm (table 5.2). Parasitism rate was higher at the high elevation farm, but significantly so only in April and May (fig. 5.5c), and was not correlated with maximum or minimum temperatures at either elevation (table 5.2). Coffee leafminer mortality due to FOPP was higher at low elevation in May, but higher at high elevation in June (fig. 5.5d), and was positively correlated with minimum temperature at the low elevation farm, but not at the high elevation farm (table 5.2).

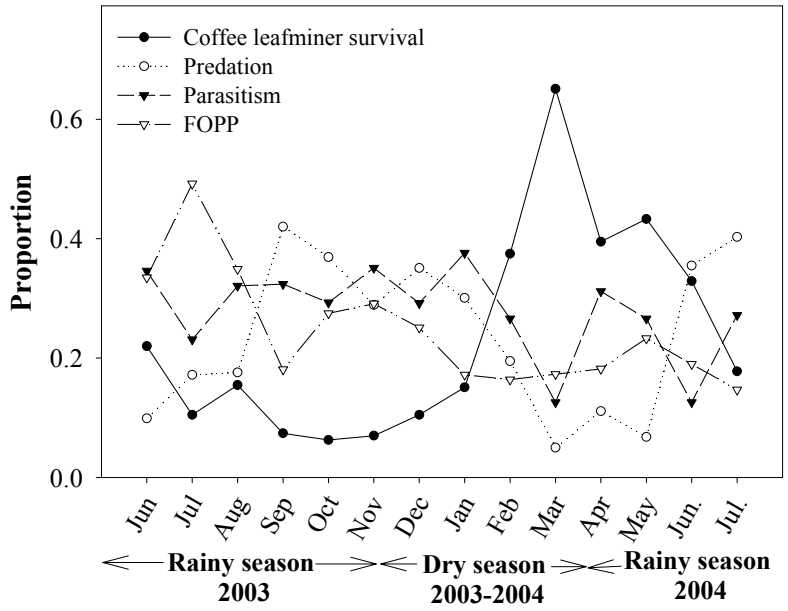

Fig. 5.3. Coffee leafminer, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), survival rate and mortality due to predation, parasitism, or factors other than parasitism or predation (FOPP) at a low elevation coffee farm (*El Encanto*, 480 m), Cacahoatán, Chiapas, Mexico, in 2003-2004.

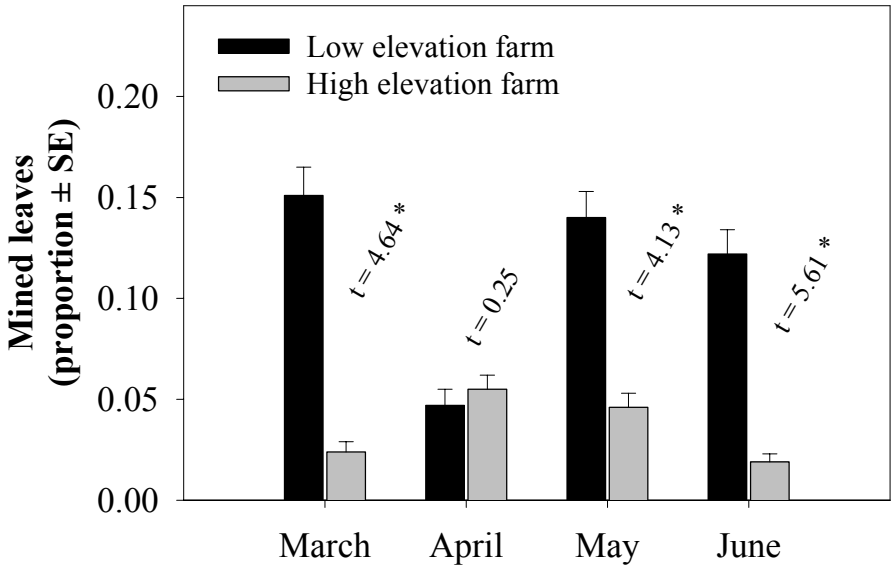

Fig. 5.4. Proportion leaves mined by coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) (± SE) at low (*El Encanto*, 480 m) and high (*Alpujarras*, 960m) elevation coffee farms, Cacahoatán, Chiapas, Mexico, in 2005. Inset statistics correspond to *t*-tests with df = 18; * indicates $P < 0.01$.

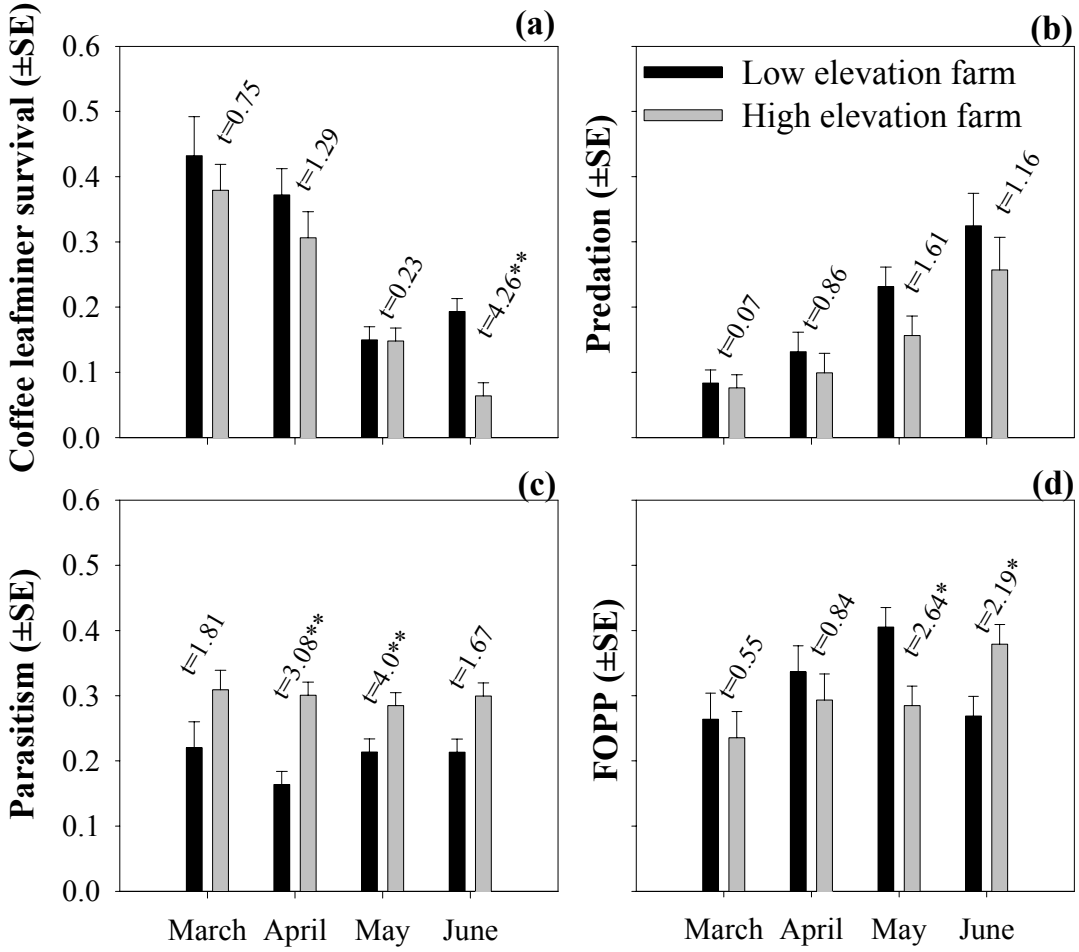

Fig. 5.5. Coffee leafminer, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), (a) larva survival rates, and larva mortality rates due to (b) predation, (c) parasitism, and (d) factors other than parasitism or predation (FOPP) at low (*El Encanto*) and high (*Alpujarras*) elevation coffee farms, Cacahoatán, Chiapas, Mexico, in 2005. Inset statistics correspond to *t*-tests with df = 18; * indicates $P < 0.01$, and ** indicates $P < 0.001$.

Table 5.2. Correlations between selected weather variables and proportion of leaves mined by coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), and coffee leafminer survival and mortality factors (predation, parasitism, factor others than predation and parasitism) at low and high elevation coffee farms, Cacahoatán, Chiapas, Mexico, March to June, 2005.

	Maximum Temperature		Minimum Temperature		Rainfall mm[1]	
Elevation/variable	r	*P*	r	*P*	r	*P*
Low elevation (El Encanto, 480 m)						
Mined leaves/mo (proportion)[1]	-0.22	0.18	-0.12	0.47	0.12	0.48
Survival rate[2]	0.47	0.002	-0.61	<0.001	-0.67	<0.001
Predation[2]	-0.59	<0.001	0.52	0.001	0.67	<0.001
Parasitism[2]	-0.15	0.36	-0.03	0.88	0.10	0.53
Other factors[2]	0.14	0.38	0.36	0.02	0.12	0.48
High elevation (Alpujarras, 960 m)[3]						
Mined leaves/mo (proportion)[1]	0.28	0.09	0.18	0.28	–	–
Survival rate[2]	0.75	<0.001	-0.27	0.10	–	–
Predation[2]	-0.55	<0.001	0.24	0.13	–	–
Parasitism[2]	-0.10	0.56	-0.19	0.25	–	–
Other factors[2]	-0.30	0.07	0.29	0.07	–	–

[1] Estimated from 10 plants/month (9 branches/plant)

[2] Estimated from 180 leaves/month

[3] Rainfall was not recorded at the high elevation farm due to calibration problems with the rain gauge

Effects of shade cover on rainfall distribution and temperature. Shade cover was negatively correlated with maximum temperature (r = -0.38; $P = 0.04$) and rainfall dispersion index (r = -0.40; $P = 0.03$). Shade cover was not significantly correlated with minimum temperature (r = -0.26; $P = 0.17$) or total rainfall (r = -0.28; $P = 0.13$).

Effects of shade cover on coffee leafminer incidence. The proportion of mined leaves during periods of low or high rainfall was not significantly correlated with shade cover ($P \geq 0.12$), but the proportion of mined leaves during the period of high rainfall was significantly correlated with minimum temperature (r = - 0.20; $P = 0.03$), but not with maximum temperature ($P = 0.94$) or rainfall ($P = 0.10$) (table 5.3).

Effects of shade cover on coffee leafminer survival and mortality due to natural enemies. Shade cover was significantly correlated with rates of larval predation (r = - 0.48; $P = 0.02$) and death by FOPP (r = 0.59; $P < 0.01$), and pupa survival (r = 0.40; $P = 0.03$); maximum temperature was significantly correlated with larval predation rate (r = 0.59; $P = 0.01$); and, rainfall dispersion index with larval predation (r = - 0.42; $P = 0.05$) and pupa survival rate (r = 0.45; $P = 0.01$) (table 5.4). Larval predation rate increased with decreasing shade cover and rainfall dispersion index, and increasing maximum temperature. Coffee leafminer larval mortality by FOPP increased with increasing shade cover. Pupa survival rate increased with increasing shade cover and rainfall dispersion index (table 5.4). Egg and larva survival, egg and pupa predation, larval parasitism, and egg and pupa mortality by FOPP were not significantly correlated with any of the weather variables that were evaluated (table 5.4).

Table 5.3. Correlations between shade cover and selected weather variables and proportion of leaves mined by coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) at a low elevation coffee farm (*La Gloria*, 550 m), Cacahoatán, Chiapas, Mexico, during periods of low and high rainfall.

	Proportion of mined leaves [1]			
	Low rainfall [2]		High rainfall [3]	
Variable	r	*P*	r	*P*
Shade cover (%)	0.14	0.12	0.04	0.65
Maximum temperature (^{o}C)	-0.14	0.12	0.01	0.94
Minimum temperature (^{o}C)	-0.02	0.85	-0.20	0.03
Rainfall (mm)	-0.11	0.24	0.15	0.10

[1] Estimated from 960 branches (8 branches/plants, n=120 plant) without coffee leafminer damage labeled in April 2005

[2] Low rainfall was <100 mm/mo (April 27-May 12, 2005)

[3] High rainfall was >400 mm/mo (June 27-July 12, 2005)

Table 5.4. Correlations between selected weather variables and coffee leafminer *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) survival and mortality rates at a low elevation coffee farm (*La Gloria*, 550 m), Cacahoatán, Chiapas, Mexico, June 22 to July 5, 2005.

Stage/variable[1]		Weather variables				
		Shade	Maximum Temperature	Minimum Temperature	Total Rainfall	Rainfall Distribution[2]
EGG						
Survival	r	0.02	-0.11	0.17	0.32	0.14
	P	0.90	0.56	0.37	0.08	0.45
Predation	r	-0.04	0.08	-0.17	-0.31	-0.16
	P	0.82	0.66	0.37	0.10	0.40
FOPP[3]	r	0.17	0.31	0.13	0.13	0.30
	P	0.36	0.10	0.51	0.48	0.11
LARVA						
Survival	r	-0.28	0.19	0.13	0.10	-0.05
	P	0.20	0.38	0.56	0.66	0.82
Predation	r	-0.48	0.56	0.11	0.15	-0.42
	P	0.02	0.01	0.63	0.49	0.05
Parasitism	r	-0.12	-0.22	0.10	0.17	-0.04
	P	0.58	0.32	0.64	0.44	0.87
FOPP[3]	r	0.59	-0.35	-0.17	-0.29	0.31
	P	0.003	0.10	0.44	0.17	0.15
PUPA						
Survival	r	0.40	-0.19	0.004	-0.10	0.45
	P	0.03	0.31	0.99	0.59	0.01
Predation	r	-0.23	0.18	0.09	0.11	-0.32
	P	0.23	0.36	0.66	0.57	0.09
FOPP[3]	r	-0.04	-0.11	-0.14	-0.02	0.05
	P	0.82	0.57	0.47	0.90	0.79

[1] Coffee leafminer survival and mortality rates were assessed in the field using coffee leafminer cohorts from a laboratory colony

[2] Coefficient of variation of daily rainfall among 4 rain gauges (= variance/mean)

[3] Factors other than parasitism and predation

Rainfall and elevation effects on coffee leafminer survival and mortality factors. Egg survival rates were higher at low versus high elevation (P <0.01), and were similar under low and high rainfall (P = 0.38) (table 5.5). Survival rates for coffee leafminer larvae and pupae did not differ significantly between elevations ($P \leq 0.16$), though they were higher under low versus high rainfall (P <0.01) (table 5.5)

Egg and larva predation rates did not differ between elevations ($P \leq 0.48$,) or rainfall levels ($P \leq 0.57$). Predation contributed >90% of total egg mortality, and >8% of larval total mortality at both elevations (table 5.5). Pupal predation rates did not differ significantly between elevations (P = 0.01) or rainfall levels (P <0.01) (table 5.5). Coffee leafminer egg or pupal parasitoids were not detected. Larval parasitism rates at low elevation contributed >40% of total larval mortality, and were ~2× greater than at high elevation (P = 0.03), but they did not differ between rainfall levels (P = 0.41) (table 5.5).

The contribution of FOPP to total mortality of coffee leafminer eggs did not differ between elevations (P = 0.99) or rainfall levels (P = 0.82), and did not exceed 6% at both elevations and rainfall levels. Larval mortality rates due to FOPP were greater at high elevation versus low elevation (P <0.01), and under high versus low rainfall levels at low elevation (P = 0.02), though at high elevation they did not differ between rainfall levels (table 5.5). Pupa mortality rates due to FOPP did not differ between rainfall levels (P = 0.11), but were higher at high elevation and low rainfall versus low elevation and high rainfall (P = 0.05) (table 5.5).

Table 5.5. Influence of elevation (low or high) and rainfall level (low or high) on coffee leafminer, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), survival rate and mortality due to predation, parasitism, or factors other than parasitism and predation (FOPP).

Elevation/rainfall [1]	Coffee leafminer survival	Contribution of natural enemies and other factors to total mortality		
		Predation	Parasitism	FOPP
(a) EGG				
Low/low	0.61±0.16(a)	0.94±0.01(a)	0	0.06±0.08(a)
Low/high	0.42±0.16(a)	0.97±0.06(a)	0	0.03±0.06(a)
High/low	0.13±0.11(b)	0.99±0.02(a)	0	0.003±0.02(a)
High/high	0.13±0.11(b)	0.99±0.03(a)	0	0.009±0.03(a)
Elevation H^2	15.54	<0.001	N/A	<0.001
P^2	<0.01	0.99		0.99
Rainfall H^2	0.77	0.05	N/A	0.05
P^2	0.38	0.82		0.82
(b) LARVA				
Low/low	0.39±0.16(a)	0.26±0.15(a)	0.44±0.17(a)	0.31±0.15©
Low/high	0.09±0.10(b)	0.08±0.09(a)	0.40±0.16(a)	0.53±0.17(b)
High/low	0.35±0.16(a)	0.10±0.10(a)	0.19±0.13(b)	0.71±0.15(ab)
High/high	0.05±0.07(b)	0.13±0.12(a)	0.14±0.12(b)	0.73±0.15(a)
Elevation H^2	1.98	0.51	4.68	9.39
P^2	0.16	0.48	0.03	<0.01
Rainfall H^2	15.36	0.33	0.68	5.27
P^2	<0.01	0.57	0.41	0.02
(c) PUPA				
Low/low	0.26±0.15(a)	0.91±0.1(ab)	0	0.09±0.1(ab)
Low/high	0.04±0.07(b)	0.97±0.06(a)	0	0.03±0.06(b)
High/low	0.31±0.15(a)	0.65±0.16(b)	0	0.35±0.16(a)
High/high	0.18±0.13(ab)	0.89±0.11(ab)	0	0.11±0.11(ab)
Elevation H^2	3.28	6.76	N/A	3.89
P^2	0.35	0.01		0.05
Rainfall H^2	8.58	8.58	N/A	2.52
P^2	<0.01	<0.01		0.11

[1] The low elevation farm was represented by *La Gloria* (550 m), and the high elevation farm by *Alpujarras* (960 m), both in Cacahoatán, Chiapas, Mexico. Low rainfall was <100 mm (April 27-May 12, 2005) and high rainfall was >400 mm (June 27-July 12, 2005)

[2] Statistics shown are from the Scheirer-Ray-Hare extension of the Kruskal-Wallis test

Temperature effects on coffee leafminer oviposition rate. Coffee leafminer oviposition rate during a 48-h period differed significantly among temperatures (P <0.01). Coffee leafminer females did not lay eggs at 20 ^{o}C, and laid significantly fewer eggs at 25 ^{o}C (3.0 ± 2.1 eggs/female) than at 28 ^{o}C (15.2 ± 5.9 eggs/female) (χ^2 = 35.82; df = 2; P < 0.01).

Circadian cycle of coffee leafminer oviposition. Average hourly solar radiation from sunset to sunrise was 0.82 ± 0.18 Watt/m^2 (range = 0.0–5.7 Watt/m^2), and from sunrise to sunset was 451.7 ± 30.24 Watt/m^2 (range = 55.9–882.9 Watt/m^2). Solar radiation (4-h average) significantly influenced coffee leafminer 4-h fecundity (r^2 = -0.60; P <0.001; n = 144). Fecundity was ~18-fold higher during the scotophase (13.4±0.6 eggs/female; range = 0-55) versus the photophase (0.8±0.6 eggs/female; range = 0-13) (χ^2 = 17.00; df = 1; P < 0.001) (fig. 5.6).

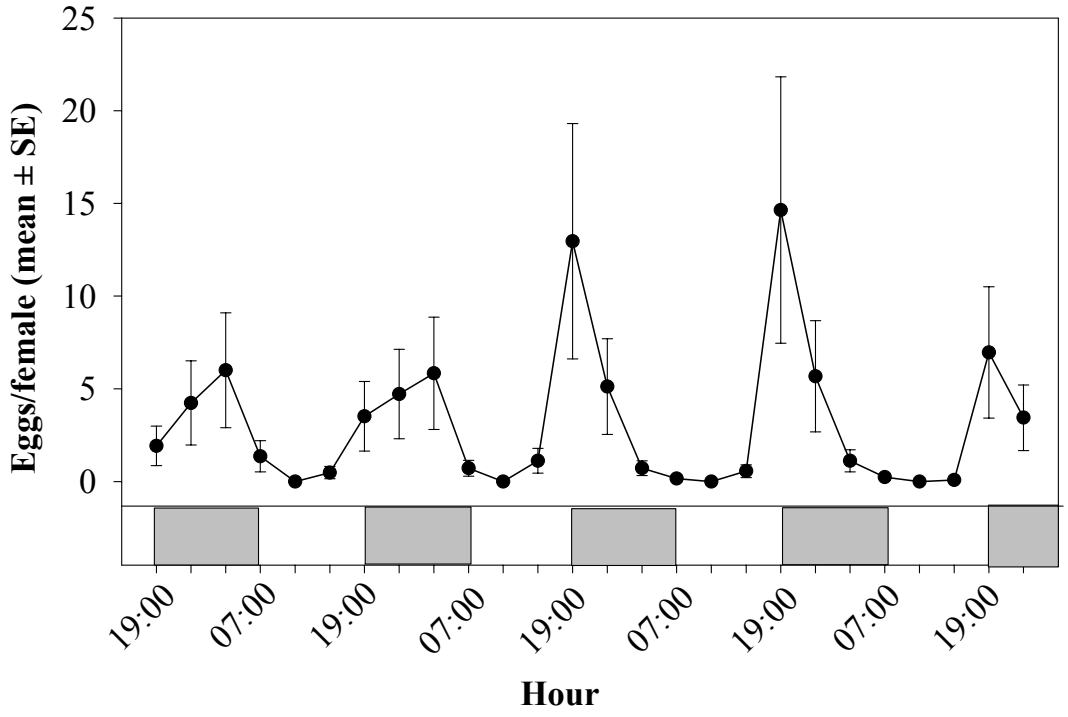

Fig. 5.6. Circadian cycle of coffee leafminer, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), oviposition in a 4 day period (mean number of eggs per 4-h period/female ± SE) between August 3 and 7, 2005. Data were obtained under laboratory conditions, 26 ± 1 ^{o}C, moisture near saturation, and natural photoperiod. Photophase and scotophase are indicated by hollow and filled portions of the horizontal bar, respectively.

Discussion

Coffee leafminer was present throughout the year in coffee farms at both low and high elevation, but its incidence was variable among months and between elevations. The proportion of mined leaves was higher during the rainy versus the dry season (fig. 5.2), and also greater at low versus high elevation (fig. 5.4), and increased with increasing minimum temperatures and rainfall, and decreased with increasing maximum temperatures. Coffee leafminer survival rate was positively correlated with maximum

temperatures (table 5.1). However, rates of predation, parasitism or death by FOPP were not significantly correlated with any of the weather variables evaluated in this study (table 5.1). Coffee leafminer survival rates were highest at the end of the dry season when predation rates were lowest (fig. 5.3). The proportion of leaves mined by coffee leafminer early in the rainy season (March-June, 2005) was greater in the low relative to the high elevation farm, except in April when the proportions were similar (fig. 5.4). Coffee plants were pruned in April in the low elevation farm, and this may explain the lower proportion of mined leaves in that month in this farm. Maximum temperatures, independently of elevation, were positively correlated with coffee leafminer survival, but not with proportion of mined leaves. This inconsistency may be a result of recording the proportion of mined leaves independently of number of mines per leaf or whether the coffee leafminer was alive or not. Maximum temperatures, independently of elevation, also were negatively correlated with predation rates, while minimum temperatures were negatively correlated with survival, and positively affected predation only at the low elevation farm. Rainfall, which was recorded only at the low elevation farm, negatively affected coffee leafminer survival and positively affected predation rates (table 5.2). None of the selected weather variables significantly impacted parasitism rates or death by FOPP (table 5.2). Field experiments with cohorts from lab-reared coffee leafminer showed that shade cover negatively affected predation of coffee leafminer larvae and positively affected pupa survival, and death of larvae due to FOPP. Experiments with coffee leafminer cohorts also showed that predation, under some conditions (e.g., low elevation independently of rainfall level, and high elevation and high rainfall level)

contributed ~90% or more of coffee leafminer egg and pupa mortality (table 5.5). At high elevation (*Alpujarras*), aside from predation, FOPP contributed more than parasitism to coffee leafminer mortality, while at low elevation parasitism was more important under low rainfall, and FOPP was more important under high rainfall (table 5.5).

The coffee leafminer population dynamics observed in this study were similar to those reported by Campos *et al*. (1989) and Flórez and Hernández (1981) in Guatemala and Colombia, respectively, but not with those reported by Villacorta (1980) and Tuelher *et al.* (2003) in Brazil, and Nestel *et al*. (1994) in Veracruz, Mexico. Higher proportions of mined leaves were observed in this study during the rainy season, relative to the dry season, and decreased with maximum temperatures and increased with rainfall, while coffee leafminer larva survival decreased with rainfall and increased with maximum temperature. Also, the proportion of mined leaves was significantly greater in low (480 m) versus high elevation (960 m) farms (fig. 5.4).

Some factors that have been suggested for explaining differences in coffee leafminer incidence between seasons and elevations include rainfall and temperature, and their effects on the coffee plant, coffee leafminer, and its natural enemies (Reis *et al*., 1976; Villacorta, 1980; Flórez & Hernández, 1985; Campos *et al*., 1989; Nestel, *et al*., 1994; Tuelher *et al.*, 2003; Guerreiro-Filho, 2006). Coffee plants, like most tropical plants, undergo significant changes in their phenology, biochemistry, and physiology with changing weather conditions (Blanco *et al*., 2003), and those changes may affect their relationships with both herbivores and natural enemies. In tropical environments,

most plant-insect studies showed that herbivores prefer young leaves over mature leaves, thus higher number of herbivores are expected when plants produce new foliage, which in the tropics typically occurs during the rainy season (Williams-Linera & Herrera, 2003). Though coffee plants produce new foliage during the rainy season (Blanco *et al*., 2003), coffee leafminer did not show oviposition preference for young leaves (Guerreiro-Filho, 2006; chapter IV). Thus, production of new foliage by coffee plants may not explain the higher incidence of coffee leafminer observed during the rainy season in the present study.

Weather variables, particularly temperature and rainfall, have been suggested as the primary factors determining abundance and distribution of coffee leafminer natural enemies (Reis *et al*., 1976; Villacorta, 1980; Gravena, 1983; Flórez & Hernández, 1985; Campos *et al*., 1989; Tuelher *et al.*, 2003; Costa-Conceiçao *et al*., 2005). However, results are contradictory between some studies. For example, Campos *et al*. (1989) suggested that the reduction of rainfall negatively affected parasitoid communities allowing coffee leafminer populations to increase during the dry season, while Flórez & Hernández, (1981) suggested negative effects of high rainfall on parasitoid host searching activity, so parasitoids did not maintain coffee leafminer at low levels during the rainy season. Results from the present study showed higher levels of mined leaves during the rainy season, in line with the results of Flórez and Hernández (1981), though parasitism rates on average were higher during the rainy season compared to the dry season.

Parasitism rates observed in the present study varied between 13% (March, 2004) and 38% (January, 2004) with an average of 27±8%. (fig. 5.3). Parasitoids were reared only from coffee leafminer larvae, and monthly parasitism rates did not explain the fluctuations in coffee leafminer survival or incidence throughout the year (fig. 5.3). The results of experiments with lab-reared coffee leafminer cohorts showed that rainfall did not affect larval parasitism, but was greater (>40%) at low versus high elevation (<20%) (table 5.5). Historical data showed that rainfall was highly variable during the year, but did not differ between elevations, while temperature was relatively constant during the year, but generally higher at low versus high elevation (fig. 1). Thus, mortality rates due to parasitism likely were more influenced by rainfall during the year, and by temperature between elevations.

Reis *et al*. (2000) suggested that predation by wasps (Vespidae) was the main source of coffee leafminer mortality in Brazil, and Tuelher *et al*. (2003) observed greater mortality of coffee leafminer larvae due to predatory wasps (Vespidae) at elevations above than below 860 m in Brazilian coffee farms. The results of the present study showed that predation rates, though not by Vespidae but by ants, were similar at both elevations (table 5.5), but during a period of low rainfall (May, 2005) was higher at the low elevation farm (fig. 5.5), and increased with rainfall and decreased with maximum temperature (fig. 5.3). Also, this study's results suggested that coffee leafminer larva survival was strongly affected by predators (table 5.2), particularly during the rainy season (~40% mortality due to predation), and less during the dry season (~5%) (fig. 5.3). Also evident was a tendency for increasing coffee leafminer egg and pupal

predation during the rainy season (table 5). Previous studies in tropical environments showed a significant increase of ant abundance during the rainy season relative to the dry season (Tanaka & Tanaka, 1982).

Ants were the predators most frequently associated with coffee leafminer in the study area (chapter III), so it is likely that ants contributed substantially to the greater predation rates evident during the rainy season. However, while predation helped to explain coffee leafminer survival during the year, and the proportion of mined leaves during the first months of the dry season, it did not explain the high incidence of coffee leafminer during the rainy season, especially during June and July, 2004, when proportions of mined leaves reached their highest level (~50%) (fig. 5.2). It is likely that the ants species found on coffee plants, which are generalist predators (chapter III), responded to coffee leafminer densities, increasing predation rates as coffee leafminer densities increased.

As noted above, temperature was likely the main weather variable behind differences in coffee leafminer abundance between elevations. Temperature affects coffee leafminer survival, oviposition, and development time. Parra (1985) established that 27 ^{o}C was the ideal temperature for coffee leafminer development and oviposition. At 27 ^{o}C coffee leafminer development time was ~2× shorter and oviposition was ~19× of that at 20 ^{o}C. In the present study, coffee leafminer did not lay eggs at 20 ^{o}C, and 48-h oviposition at 25 ^{o}C was significantly lower (~3 eggs/female) than at 28 ^{o}C (~28 eggs/female). Average temperature at the high elevation farm (*Alpujarras*) was ~21 ^{o}C (range = 13.5-28.5 ^{o}C), while at the low elevation farm (*El Encanto*) was ~25 ^{o}C (range

= 18.0-32.0 ^{o}C). Coffee leafminer oviposits mostly during the night (fig. 5.6), which is when minimum temperatures generally occur in the area of study. Overall, the results suggested that differences in incidence and abundance of coffee leafminer between elevations were primarily due to differences in temperature, rather than rainfall, because low temperatures at high elevation likely reduced coffee leafminer oviposition and fecundity, and may have increased its mortality rate as a consequence of a longer development time and exposure to natural enemies (Fordyce & Shapiro, 2003; McMillan *et al*., 2005).

Major outbreaks of coffee leafminer in the Neotropics are reportedly associated with elimination or drastic reduction of shade cover from coffee farms (Guharay *et al.* 2001; Monterrey *et al.,* 2001; Fragoso *et al*., 2002; Carvalho *et al*., 2005). Some authors found that shade cover modified micro-environmental conditions, reducing direct impacts of rainfall, temperature, evapotranspiration, and total radiation (Barradas & Fanjul, 1986; Guharay *et al*., 2001), which may affect coffee leafminer mortality rates and natural enemies. For example, Philpott (2005) recorded important reductions in ant abundance and diversity after coffee shade-trees were pruned in Chiapas, Mexico. The present study showed that while increasing shade cover reduced temperatures and modified the local distribution of rainfall within the farm, shade cover was not correlated with the proportion of mined leaves or coffee leafminer survival. However, a severe reduction of shade cover may lead to warmer temperatures, and to acceleration of coffee leafminer development and enhancement of its fecundity.

Overall, the results of this study showed that coffee leafminer was present throughout the year in coffee farms at low and high elevations, but its incidence was variable among months and between elevations. Weather variables had important effects on coffee leafminer phenology: the proportion of leaves mined by coffee leafminer increased with rainfall, and decreased with maximum temperatures, and was higher during the rainy versus the dry season, and at low versus high elevations. Predation was the main source of coffee leafminer mortality, and during the rainy season high levels of coffee leafminer incidence coincided with high rates of predation. The effect of temperature on coffee leafminer oviposition rate, and its marked circadian oviposition cycle, may explain in part differences in coffee leafminer incidence between low and high elevation farms. Shade cover reduced farm-level temperatures, but did not affect coffee leafminer incidence. Although the present study was useful for understanding the contributions of temperature to differences in the incidence of coffee leafminer between elevations, further and more detailed studies are needed, including field experiments addressing coffee leafminer mortality via life tables and sentinels at more elevations, and over longer periods of time. Also, laboratory and field experiments are needed addressing effects of intra-guild predation by parasitoids and predators, parasitoid host feeding, and leaf damage by predators on coffee leafminer larval direct and indirect mortality.

CHAPTER VI

CONCLUSION

Overall, the results of field surveys and laboratory experiments suggested that coffee leaf nitrogen content (percentage of dry weight) and leaf penetrability index significantly impacted the incidence and development of coffee leafminer, though the ovipositional preference of adult females was not generally for leaves that maximized offspring performance. Life table analyses showed that coffee leafminer populations were negatively affected by a highly diverse complex of natural enemies in the Soconusco area of Chiapas, Mexico. Daily minimum temperatures below 20 ^{o}C likely reduce the reproductive potential of coffee leafminer. Thus the presence of natural enemies and low temperatures were the factors that explained most of the variation in coffee leafminer abundance and distribution in the study area. Those results suggested that top-down and abiotic factors, more than bottom-up factors, such as host plant attributes, significantly influenced coffee leafminer densities in the two localities studied in the Soconusco region of Chiapas, Mexico.

Coffee plants in two Soconusco region farms were highly variable in the physical (leaf size, penetrability, specific weight) and chemical (leaf nitrogen content) properties evaluated, as well as in levels of coffee leafminer incidence. These results permitted exploration of host plant effects on coffee leafminer distribution and abundance according to the central prediction of the *optimal oviposition behavior hypothesis* that herbivores should oviposit on host plants that maximize offspring performance (Jaenike,

1978). Thus, coffee leafminer was expected to occur more frequently in young and unmined leaves, leaves with high leaf nitrogen content, and in plants with large leaves, as found in other herbivore insects (Pfeiffer & Burtd, 1983; Craig *et al*., 1989; Price, 1991; Ateyyat & Mustafa, 2001; Heisswolf *et al*., 2005). Also, greater coffee leafminer survivorship and growth were expected on leaves with high nitrogen content, high specific weight, and low leaf penetrability index (Stamp & Bowers, 1990; Leather, 1994; Wheeler & Center, 1996; Awmack & Leather, 2002). The results of laboratory experiments presented in chapter II showed that coffee leaf quality significantly affected coffee leafminer survivorship, growth, and development time, though not as expected. Leafminers reared on leaves with moderate nitrogen content (2.9±0.01%) had shorter developmental time and grew larger than those reared on leaves with low (2.5±0.04%) or high (3.4±0.01%) nitrogen content. Leafminers reared on tough leaves (penetrability = 101.2±0.8 g/mm^2) had higher survival rates and were larger than those reared on soft leaves (66.5±0.6 g/mm^2). However, coffee leafminer females oviposited indiscriminantly among leaves in the laboratory, rather than on leaves that maximized the offspring performance. These results suggested that coffee leafminer abundance and distribution in the field is not determined solely by plant quality, but also by other factors, including natural enemies and physical environmental variables, which were examined in chapters III, IV and V.

Studies on coffee leafminer mortality factors conducted in the Neotropics suggested that coffee leafminer population dynamics were strongly affected by both natural enemies (Flórez & Hernández, 1981; Gravena, 1983; Campos *et al*., 1989; Paliz

& Mendoza, 1993), and physical environmental conditions prevalent at different elevations (e.g., de Souza, 2003). The results presented in chapter III showed that coffee leafminer egg to adult mortality was lower at low (78-89%) versus high (96-97%) elevation, and that, independently of elevation or rainfall level, egg and pupal predation, and death of larvae due to factors other than predation and parasitism were the main causes of coffee leafminer mortality.

Typically, leafminers have a rich community of associated natural enemies (Hespenheide, 1991; Whitfield & Wagner, 1991; Rott & Godfray, 2000), and the results presented in chapters III and IV showed that coffee leafminer was not an exception. The natural enemy complex of coffee leafminer in the study area included at least 22 parasitoid and 16 predator species. A field-study with laboratory-reared coffee leafminer cohorts showed that parasitism rates were lower than predation rates (chapter III), though parasitism rates may have been underestimated, because mortality rates ascribed to factors other than predators and parasitoids (FOPP), which in some months represented ~50% of total mortality, likely included parasitoid host feeding and leaf damage by predators, which expose coffee leafminer larvae to adverse weather conditions and pathogens.

The parasitoid list compiled in this study included eight species recorded for the first time associated with coffee leafminer in Mexico, and 3 new records for coffee leafminer for the Neotropics (*Aprostocetus* sp., *Neochrysocharis arvensis* Graham, and *N. chalybea* Hansson, all three Eulophidae). Parasitism in two coffee farms studied in the Soconusco region of Chiapas accounted for <10% of real mortality, and was higher

at low (8-10%) versus high (0.8-1.0%) elevation. The parasitoid species complex was composed entirely of larval parasitoids; egg and pupal parasitoids were not recovered.

The coffee leafminer predator complex was composed mainly (~88%) of ants (Formicidae), most of them (93.3%) representing new records for coffee leafminer. At least four ant species in four genera were observed preying on coffee leafminer eggs, four in two genera feeding on larvae, and six in four genera on pupae. Not all of the 13 ant species that were collected were identifiable with existing keys, and were sent for identification to a specialist. *Camponotus* sp. 1, *Pseudomyrmex* sp. 3, *Pseudomyrmex* sp. 2, and *Azteca* sp. were the ant species most frequently observed preying on coffee leafminer. The results of the present study contrast those of Reis *et al*. (2000) who found that wasps (Vespidae) were the principal coffee leafminer predators in Brazil. Predatory ants may be ecologically equivalent in Soconusco coffee farms to vespid wasps in Brazilian farms, because only once was a predatory wasp observed preying on coffee leafminer larvae in numerous field observations in the Soconusco region between 2003 and 2005 (chapter III), including 32 h of observation on sentinels, in contrast with 78 predation events by ants during observation on sentinels. However, it is unclear whether studies conducted on predation of coffee leafminer in Brazil, or elsewhere, considered or focused on ants. Moreover, the differences in predator species composition also could be due to management practices, because Brazilian coffee production is mainly under sun conditions and farms are intensively managed, while Chiapas production is under shade conditions and farms are traditionally managed.

Ants were the most frequent predators of coffee leafminer eggs and pupae, which supports hypotheses pointing to ants as the most important mortality factor affecting herbivore populations in tropical systems (e.g., Way & Khoo, 1992). Some studies suggested eliminating ants to enhance biological control (Reimer *et al*., 1993; Infante *et al*., 2003), while others pointed to enhancing ant populations to increase predation rates and reduce pest population densities (Philpott & Foster, 2005). Because of such conflicting recommendations, it will be necessary to carefully quantify the impacts of individual ant species on coffee leafminer populations, as well as on the populations of other coffee herbivores, to fully ascertain the potential role of ants in coffee IPM programs.

Various studies suggested that weather variables, particularly temperature and rainfall, were the primary factors determining abundance and distribution of coffee leafminer and its natural enemies (Reis *et al*., 1976; Villacorta, 1980; Gravena, 1983; Flórez & Hernández, 1985; Campos *et al*., 1989; Tuelher *et al.*, 2003; Costa-Conceiçao, 2005). The results presented in chapter V showed that while coffee leafminer was present throughout the year in coffee farms at low and high elevations, its incidence was variable among months and differed between elevations. The proportion of leaves mined by coffee leafminer was positively correlated with rainfall and negatively with maximum temperatures. The proportion of mined leaves was higher during the rainy versus the dry season, and also at low versus high elevation, while predation rates were highest during the rainy season, coinciding with the highest levels of coffee leafminer incidence.

Analysis of historical weather data showed that monthly rainfall patterns were likely similar at the low and high elevation farms studied, though average temperature at the high elevation farm was slightly cooler, ~21 ^{o}C (range = 13.5-28.5^{o} C), compared to the low elevation farm, ~25 ^{o}C (range = 18.0-32.0 ^{o}C). Parra (1985) indicated that 27 ^{o}C was the ideal temperature for coffee leafminer development and oviposition. Put together, some results of the present study suggested that differences between elevations in incidence and abundance of coffee leafminer, and in coffee leafminer mortality sources were likely related more to differences in temperature, rather than rainfall, between elevations. Specifically: (i) coffee leafminer incidence in the field was usually lower at high compared to low elevation, and where temperature averages and minima were lower; (ii) in the laboratory, coffee leafminer oviposition was nil to minimal at 25 ^{o}C or below, and temperatures in that range occurred more frequently at high compared to low elevation, and; (iii) practically all oviposition occurred during the night, which is when temperature minima occur in the field. Thus, it is plausible that temperatures below 25 °C, which occur more frequently at high compared to low elevation, reduce coffee leafminer's reproductive rate, and may increase its mortality rate as a consequence of longer developmental times and greater exposure to natural enemies. Although higher mortality rates have been associated with slower development in various insects (Fordyce & Shapiro, 2003), such an outcome has not been demonstrated in the case of coffee leafminer, so merits experimental testing.

Though more study is warranted, the results form this study allow making some general recommendations. Coffee leafminer's reproductive potential is greatest at 27 °C,

and below 20 °C its reproductive potential is near nil. Thus, new coffee farms should be planted in areas where temperature minima frequently are below 20 °C, which also is inside the optimal temperatures for growing arabica coffee recommended by Enriquez (1993). Moreover, monitoring coffee leafminer incidence is very important, especially following the winter season, when coffee leafminer abundance increases in the study area. Conservation and increase of ant species especially of *Pseudomyrmex* (which are known to tend homopteran pests) may contribute to suppressing coffee leafminer populations.

Overall, the results of field and laboratory research showed that coffee leafminer had a high diversity of associated natural enemies, and suggested that the abundance and distribution of coffee leafminer was not strongly associated with adult ovipositional preferences. Then, differences in the incidence of coffee leafminer among months and between low and high elevations were most likely due to differences in predation rates and temperatures between elevations. However, this conclusion should be verified further through additional manipulative field studies. For example, through studies employing laboratory-reared coffee leafminers that compare the impacts of weather variables on coffee leafminer abundance and distribution, and survival and mortality factors acting on coffee leafminer eggs, larvae, pupae, and adults, among a series of elevations and months. This is particularly important because most of the studies conducted to date focused only on the larval stage, and were conducted over a limited number of months (Flórez & Hernández, 1981; Gravena, 1983; Aranda-Delgado, 1986; Campos *et al*., 1989; Paliz & Mendoza, 1993). In addition, “exclusion experiments,”

such as those conducted by Fowler & MacGarvin (1985), which examined predation on leafminers, their natural enemies, and other herbivores, by generalist ant predators on birch trees, should provide useful information concerning the impacts of ants on coffee leafminer, its natural enemies, and other coffee herbivores. Additional studies, similar to those conducted by Philpott & Foster (2005), who artificially increased ant-nest numbers on coffee plants, and involving coffee leafminer sentinels, will provide information on the impacts of ant diversity and abundance on coffee herbivores, including coffee leafminer. Field and laboratory experiments should document and quantify any impacts of intra-guild predation**,** including predation of parasitoids by ants, and of parasitoid host feeding on coffee leafminer population dynamics and abundance. Finally, studies on the effects of temperature and protracted coffee leafminer development on parasitism and predation rates should test the hypothesis that longer developmental time does not affect coffee leafminer mortality due to natural enemies.

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